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# ON THE NATURE OF THE CONDITIONS WHICH DETERMINE OR PREVENT THE ENTRANCE OF THE SPERMATOZOON INTO THE EGG

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## I

THE well-known fact that a spermatozoon can no longer enter an egg after it is once fertilized raises the question whether this is due to the changes necessarily connected with development; or whether development of an egg can take place without the existence of such a block. We are in possession of facts speaking in favor of the second view. Thus the writer has shown that if the eggs of *Strongylocentrotus purpuratus* or *Arbacia* are induced to develop by the methods of artificial parthenogenesis a spermatozoon can enter the egg or an individual blastomere of a segmenting egg, while the latter is in the full process of development. This leaves no doubt that the block caused by the entrance of a spermatozoon into an egg for the entrance of further spermatozoa must be due to a change not necessarily identical with that inducing the development of the egg.

A second group of observations made by the author deals with the phenomena of specificity and these prove that the block which an egg offers to heterogeneous sperm is rapidly reversible and confined to the surface of the egg or the spermatozoon or both. In the case of the egg of *purpuratus* and sperm of *Asterias* (and many similar in-

stances) the specific block can be overcome if we slightly increase the alkalinity of the sea water. The spermatozoon can only enter the foreign egg while both sperm and egg are in the hyperalkaline sea water, whereas if the egg and sperm are treated separately with hyperalkaline solution (no matter how long) and put together in a sufficiently large quantity of normal sea water no egg can be fertilized[1] while fertilization will take place as soon as the hyperalkalinity is restored. This shows that the change (brought about by the hyperalkaline sea water) which makes the fertilization possible is rapidly reversible, as we should expect it to be if it consisted merely in a physical change at the surface of the egg. To this series of facts, others might be added which point in the same direction. In this paper we intend to discuss a little more fully the various conditions which block or favor the entrance of a spermatozoon into an egg, in order to form an idea of the nature of the forces which control these phenomena.

## II

1. When the unfertilized eggs of *S. purpuratus* are treated for two hours with hypertonic sea water (50 c.c. sea water + 8 c.c. 2½ *m* NaCl or Ringer solution) the eggs of certain females will develop into blastulæ, gastrulæ and plutei, while the eggs of other females can not be caused to develop in this way. These individual differences coincide possibly with those observed by the writer in regard to spontaneous membrane formation in the eggs of different females[2] and it is possible that only the eggs of such females of *purpuratus* can be induced to form larvæ through a mere treatment with hypertonic sea water in which the latter can induce the cortical changes underlying the membrane formation. Whatever the nature of the individual difference may be, *purpuratus* eggs

[1] The large quantity of sea water is necessary so that the hyperalkaline sea water at the surface of the egg and sperm can diffuse away before both gametes come in contact.

[2] Loeb, *Arch. f. Entwcklngsmech.*, XXXVI, 626, 1913; "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 219.

which have been induced to develop into larvæ by a hypertonic solution can be fertilized with sperm while they are in the process of segmentation. When such eggs are in the two-, four-, eight-, or sixteen-cell (and possibly also later) stages the sperm can enter into one or more blastomeres of such an egg and this entrance betrays itself by a distinct and clear membrane formation around each blastomere.[3] While the segmenting eggs which were not fertilized with sperm develop into larvæ, those into which sperm enters perish very rapidly. This simple and rather striking experiment which can easily be performed in the eggs of *Strongylocentrotus,* where the membrane formation around a single blastomere can be clearly recognized, shows that the process of development in a fertilized egg in itself can not be responsible for the block caused by fertilization. It looks as if the entrance of a spermatozoon into the mature egg, independently of the developmental changes it induces in the egg, causes some physical or physico-chemical change (of the surface of the egg?) which renders the subsequent entrance of a spermatozoon impossible.

2. With the eggs of most females of *purpuratus* the treatment with a hypertonic solution does not lead to a development into larvæ, but only to the first segmentation stages in a limited number of eggs (provided that the eggs have been exposed to the solution the proper period of time). Such blastomeres afterwards go into a resting stage. If one waits long enough, until there is no doubt left that the blastomeres have reached a resting condition and will divide no further, and if one then adds sperm, the individual blastomeres can again be fertilized, which is indicated by a membrane formation around each individual blastomere and the subsequent development of such blastomeres into swimming larvæ.[4] The fact that each individual blastomere in this case is fertilized independ-

[3] Loeb, "Artificial Parthenogenesis and Fertilization," p. 240; *Arch. f. Entwcklngsmech.,* XXIII, 479, 1907.

[4] Loeb, *Arch. f. Entwcklngsmech.,* XXIII, 479, 1907; "Artificial Parthenogenesis and Fertilization," p. 237.

ently of its neighbors suggests that there is no protoplasmic connection between the neighboring blastomeres; otherwise the entrance of a spermatozoon into one should cause its neighbors also to form a fertilization membrane, which does not happen.

All these facts show that the changes underlying development do not necessarily prevent the entrance of a spermatozoon into an egg fertilized by sperm.

3. Development can be initiated in an unfertilized egg by causing a membrane formation by a fatty acid. Eggs after such an artificial membrane formation perish as a rule rapidly at room temperature (if no second treatment is given them) but they may segment if kept at a low temperature. The eggs are usually put after treatment with the butyric acid into normal sea water in which they form a membrane. This membrane is different in the eggs of different species of sea urchins. In the egg of *S. purpuratus* the membrane is tough and entirely impermeable to the spermatozoon. When we add sperm to such eggs with a butyric acid membrane they behave exactly as if no sperm had been added, they all perish rapidly (at room temperature). The question arose, if a spermatozoon could still enter the egg of *purpuratus* after membrane formation, provided the membrane could be destroyed. This can be done in a certain percentage of the eggs of *purpuratus* by shaking them after artificial membrane formation; the number of eggs whose membrane is torn varies in different experiments owing probably to differences in the thickness and toughness of the membrane. Even if the membrane is torn the edges may come close together again so that the opening often is closed again and no spermatozoon can go through. Kupelwieser and the writer performed this experiment on the eggs of *purpuratus* and it was found that such eggs with torn membranes were fertilized upon the addition of sperm and developed normally; while the eggs whose membranes were intact all perished.[5]

[5] Loeb, "Artificial Parthenogenesis and Fertilization," p. 234.

The writer repeated this experiment last winter with the same result. He found that eggs with torn membranes when subsequently fertilized with sperm did not form any new membranes as he had stated before. It is possible that he mistook at that time the new hyaline membrane which forms around the egg after membrane formation and fertilization for a new fertilization membrane.

It is not necessary that these eggs be fertilized immediately after the artificial membrane formation, the experiment succeeds also after some time (one hour or more); only with this difference that the eggs perish very rapidly after the membrane formation if they receive no second treatment. In order to avoid this difficulty the writer last winter proceeded as follows: Artificial membrane formation was produced in the eggs of a *purpuratus* and all eggs had formed perfect membranes. One control was kept and the rest were shaken. These were divided into three lots, one served as a control; the eggs of the latter all perished as fast as the eggs of the first control (which were not shaken). The second lot were fertilized after about one half hour after membrane formation. Twenty per cent. of these eggs developed into normal larvæ, the rest perished. The percentage of developing eggs corresponded roughly with the percentage of eggs whose membrane was torn. The third lot of the shaken eggs was put overnight into 50 c.c. sea water + 7 drops of 1/10 per cent. KCN, to prevent the disintegration of these eggs. The next morning (sixteen hours after the membrane formation) the eggs of Lot 3 were transferred into normal sea water and divided into two lots, one was fertilized with sperm, the other was kept as a control. About twenty per cent. of the eggs which were fertilized began to segment, but many in an abnormal way and none developed into larvæ. Of the second lot to which no sperm was added also a few began to segment. As the writer has shown in former experiments, the eggs of *Strongylocentrotus* can be caused to develop after artifi-

cial membrane formation if they are either treated for a short time with a hypertonic solution or if for a longer period the oxidations are suppressed in them by lack of oxygen or the addition of cyanide. There is therefore no doubt that the eggs of *purpuratus* in which the artificial membrane formation has been induced by butyric acid can be fertilized subsequently with sperm.

4. The treatment of the eggs of *Arbacia* with butyric acid leads to the formation of a membrane which varies considerably in the eggs of the same female. Some eggs have a thin membrane which is permeable to the spermatozoon, others have a tough fertilization membrane which is as impervious to the spermatozoon as the regular fertilization membrane. The percentage of the eggs with membranes permeable for sperm varies very much in different experiments, according to the material and according to the external conditions. If this is kept in mind it is easily understood that the number of *Arbacia* eggs which can be fertilized after they have been treated with butyric acid differs in different experiments. Since the membrane called forth by butyric acid is not always plainly visible, it is a prerequisite that always one set of such eggs should be set aside as controls to ascertain whether or not *all* the eggs disintegrate rapidly (if no second treatment is given to them). Only if they all disintegrate rapidly have we any guarantee that in all of them the membrane formation has been effective. The former experiments of the writer show that such eggs can be fertilized by sperm; in fact they show that while the unfertilized eggs disintegrate rapidly after the inducement of the membrane formation with butyric acid, the subsequent fertilization of such eggs by sperm saves their lives and makes them develop.[6]

## III

1. It is a well-known fact that most eggs can only be fertilized by sperm of their own or a closely related species. The writer thought that in order to obtain light

[6] Loeb, *Arch. f. Entwcklngsmech.*, XXXVIII, 416, 1914.

on the nature of the block to the entrance of heterogeneous sperm it was necessary first to find the means by which this block could be overcome. He succeeded in showing that the egg of the sea urchin *S. purpuratus* can be fertilized by the sperm of starfish, brittle stars, and holothurians in sea water (or other balanced solutions) if their alkalinity was a trifle higher than that of ordinary sea water (*e. g.*, in a solution of 50 c.c. sea water + 0.6 c.c. *N*/10 NaOH).[7] Godlewski[8] succeeded by the same method in the fertilization of the egg of the sea urchin with the sperm of crinoids

The most important fact found out in this connection was the following, namely, that the fertilization of the egg of *purpuratus* by the sperm of *Asterias* only takes place while both eggs and sperm are in this hyperalkaline solution. If eggs and sperm are put into these solutions separately and if then from time to time sperm and eggs so treated are transferred into normal sea water, as a rule not a single egg is fertilized; while with the same material when eggs and sperm are together in the hyperalkaline solution as many as 100 per cent. of the eggs may be fertilized. The effect of the alkali is, therefore, rapidly reversible; the eggs when put from the hyperalkaline sea water free from sperm into the normal sea water containing very motile sperm of *Asterias* can not be fertilized; when put back into hyperalkaline sea water containing *Asterias* sperm they will be fertilized rapidly.

This rapid reversibility of the effect of the NaOH indicates that it must be confined to the surface of the egg and the spermatozoon or both; and this is corroborated by the fact that the NaOH does not enter the cells. One of the forces which determine the entrance of the spermatozoon into the egg may be surface tension and the phenomenon of the entrance may be comparable or possibly identical with the phenomenon of phagocytosis.

Godlewski mentioned that he occasionally observed a

[7] Loeb, *Pflüger's Arch.*, IC, 323, 1903; CIV, 325, 1904; *Arch. f. Entwcklngsmech.*, XL, 310, 1914; *Science*, N. S., XL, 316, 1914.

[8] Godlewski, *Arch. f. Entwcklngsmech.*, XX, 579, 1906.

fertilization of the egg of the sea urchin with the sperm of a crinoid in normal sea water after both had been treated with hyperalkaline sea water separately. This observation is correct but finds its explanation in the assumption that in such cases the hyperalkaline sea water had not had time to diffuse from the jelly of the egg or from the surface of the egg protoplasm by the time the spermatozoon came in contact with it. In order to test this view the writer treated the eggs of *purpuratus* with a hyperalkaline solution of greater than the optimal concentration while the sperm was treated separately with the optimal concentration (50 c.c. sea water + 0.6 c.c. *N*/10 NaOH) and then both were mixed in a little sea water in a watch glass. In such a case a large number of eggs were fertilized, but while the fertilization occurred nominally in normal sea water it really occurred in a layer of hyperalkaline sea water surrounding the protoplasm of the egg.

The conclusion from these experiments is that the block to the entrance of the spermatozoon of *Asterias* into the egg of *purpuratus* is of a rapidly reversible character, consisting in some alteration of a physical property of the surface. On this assumption the factor of specificity consists of an agency which affects these properties of the surface of the egg in the same sense as the increase in the concentration of the alkali. It should be added that the writer observed also that an increase of the concentration of Ca in the sea water acts in the same sense as an increase in the alkalinity; and that if the concentration of Ca is increased the increase of NaOH may be less than is necessary otherwise.

2. If the idea was correct that the factor of specificity contained in the spermatozoon affected only the forces acting at the surface of the egg; and that the lack of this factor could be replaced by a rise in the alkalinity of the sea water, it was to be expected that the reverse should also be possible: namely, that a change in alkalinity or the constitution of the surrounding medium should pro-

duce a reversible block to the spermatozoa of the same species. That means, it should be possible to find solutions in which the egg does not suffer for a long time, in which the sperm lives for a long time, and in which the sperm of the same species is intensely active and attacks the egg with the greatest eagerness and yet is not able to enter; while if the medium is but slightly changed the sperm enters the egg at once. The writer carried out such experiments a year ago in Pacific Grove and last summer in Woods Hole and found this to be true.[9]

For the purpose of these experiments the ovaries and testes of the sea urchins were not put into sea water but into a pure $m/2$ NaCl solution (after several washings in such a solution) and kept in such a solution. Several drops of sperm and one drop of eggs were in one experiment put into 2.5 c.c. of a neutral mixture of $m/2$ NaCl and 3/8 $m$ $MgCl_2$ in the proportion in which these two salts exist in the sea water. In such a neutral solution no egg of *Arbacia* or of *purpuratus* is fertilized no matter how long they remain in the solution, although the sperm is very active. If the eggs and sperm are transferred into the same solution which contains in addition 1 drop of a $N/100$ solution of NaOH (or $NH_3$, or benzylamine, or butylamine) or 8 drops of $m/100$ $NaHCO_3$, most and often practically all the eggs at once form fertilization membranes and begin to segment at the proper time.

The same result can be obtained if the eggs are transferred into a neutral mixture of $NaCl + MgCl_2 + CaCl_2$ (in the proportion in which these salts exist in the sea water) or into a neutral mixture of $NaCl + MgCl_2 + CaCl_2 + KCl$. In such a neutral mixture the eggs form fertilization membranes and begin to segment.

The eggs will not be fertilized if transferred into a neutral solution of NaCl or of $NaCl + KCl$.

It is, therefore, obvious that if we diminish the alkalinity of the solution surrounding the egg and if we deprive this solution of $CaCl_2$ we establish the same reversible

[9] Loeb, *Science*, N. S., XL, 316, 1914.

block to the entrance of the spermatozoon of *Arbacia* into the egg of the same species as exists for the entrance of the sperm of starfish into the egg of *purpuratus* in normal sea water.

Another form of the experiment may be mentioned. When we put sperm and eggs of *Arbacia* (which had been washed in an *m*/2 NaCl solution) into a neutral mixture of NaCl + KCl no egg can be fertilized although the sperm may be so active and concentrated that the eggs roll around in the solution and the chorion (the jelly surrounding the egg) may be filled with spermatozoa. In one experiment the eggs and sperm of *Arbacia* were kept overnight in watch glasses containing 2.5 c.c. of this mixture of neutral NaCl + KCl. The next morning all the eggs were intact and not a single one was fertilized. At that time 20 drops of sea water were added to the mixture and instantly fertilization membranes were formed and practically all the eggs segmented.[10]

It can be shown that in this experiment the sea water added two important substances, Ca and NaOH. If NaOH alone is added to the mixture of NaCl + KCl, as a rule no egg is fertilized or only a few; if $CaCl_2$ is added to a neutral mixture of NaCl + KCl a number of eggs are fertilized. If both $CaCl_2$ and NaOH are added in the proper proportion as a rule all the eggs are fertilized.

It is perhaps important to call attention to the fact that if eggs of *Arbacia* are fertilized in sea water and if after repeated washings in a mixture of NaCl + KCl or of NaCl + $MgCl_2$ they are put into these solutions they will segment repeatedly in these solutions, thus showing that the eggs were really not fertilized in these two solutions in the above-mentioned experiments.

The striking fact is again that the block created by the

[10] This experiment was carried out with different concentrations of sperm and it was found that only in the dishes where the concentration of sperm was sufficiently high were all the eggs fertilized upon the addition of sea water. This is perfectly natural as the majority of spermatozoa die gradually (as do also the eggs) and hence enough spermatozoa will only be alive the next day if the concentration of sperm was not too low.

lack of $CaCl_2$ or NaOH or both to the entrance of the spermatozoon is removed immediately after these substances are added. The block must be due merely to a change in the physical condition of the surface (which may be based on a rapidly reversible chemical reaction).

In these experiments the NaCl can not be replaced by isotonic sugar solutions. The same fact was found by the writer to be true for heterogeneous hybridization.

It is of importance to call attention to the fact that the abolition of the block in the case of heterogeneous hybridization depends upon the same substances, $CaCl_2$ and NaOH (or some other alkali), which make normal fertilization possible. The influence of electrolytes on the fertilization of the egg of *purpuratus* by the sperm of *Asterias* is parallel to the influence of the same electrolytes on the fertilization of the same egg by the sperm of *purpuratus;* only the concentrations differ, and always in the same sense. The forces at work are, therefore, apparently the same in both cases; but we can only express surmises as to their nature. The rôle of salts as well as the rapid reversibility indicate that they are forces situated at the surface of the egg and the spermatozoon or both. In the first place we may think of surface tension conditions and in this respect it is possible that the entrance of the spermatozoon into the egg may be determined by such forces in a way similar to the process of phagocytosis. In the second place it may be that previous to the action of surface tension forces an alteration in the degree of fluidity of the egg surface may be required (*e. g.,* that physical change which finds its expression in the formation of the fertilization cone). Thirdly, it may be possible that before the surface tension forces can act the spermatozoon must agglutinate with the egg surface and that this agglutination is determined by certain specific substances or by certain salts ($CaCl_2$ and NaOH) or by both.

Brief mention should be made of the block discovered by Godlewski[11] to the entrance of a spermatozoon into

the egg if the sperm of the same species is mixed with the sperm or the blood of a species widely apart. If, for instance, the sperm of a sea urchin is mixed with the sperm of certain annelids (*Chætopterus*) or molluscs and if after some time the eggs of the same sea urchin are added to the mixture of the two kinds of sperm no egg is fertilized. If the solution is, however, subsequently diluted with sea water or if the egg that was in this mixture is washed in sea water, the same sperm mixture in which the egg previously remained unfertilized will now fertilize the egg. From these and similar observations Herlant[12] draws the conclusion that the block existed at the surface of the egg, inasmuch as a reaction product of the two types of sperm is formed after some time which alters the surface of the egg and thereby prevents the sperm from entering. This view is not only supported by all the experiments but also by the observation of the writer that foreign sperm or blood is able to cause after some time a real agglutination if mixed with the sperm of a sea urchin or a starfish.[13] We can imagine that the precipitate forms a film around the egg and acts as a block which can be removed mechanically by washing.

It is not impossible that the block which exists in the fertilized egg is due also to an alteration of the physical character of the surface of the egg which in this case is, however, induced from within the egg by changes caused by the entrance of the spermatozoon, which, however, are not necessarily identical with those causing development as was shown by the facts in the second chapter.

## IV.

We will now turn to the question whether the motility of the spermatozoon plays no other rôle than to bring the spermatozoon so close to the surface of the egg that surface tension phenomena can engulf the spermatozoon into the egg. It is easy to show that if the spermatozoa

[11] Godlewski, *Arch. f. Entwcklngsmech.*, XXXIII, 196, 1911.

[12] Herlant, *Anat. Anzeiger*, XLII, 563, 1912.

[13] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

of *purpuratus* are immobilized by NaCN no egg of the same species can be fertilized, no matter how concentrated the sperm; while the same sperm when it revives from the effect of NaCN fertilizes the same eggs at once. This meets with the possible objection that the motility of the sperm might be only necessary to allow the latter to penetrate the jelly surrounding the egg protoplasm. In order to test this objection the writer freed the eggs of *purpuratus* from this jelly by treating them for two minutes in a mixture of 50 c.c. sea water + 3 c.c. $N/10$ HCl in which all the jelly is dissolved. The eggs were washed afterwards in sea water and it was found that if sperm was added practically all were fertilized. The writer put such eggs with sperm which was immobilized by NaCN. The eggs and the sperm were squirted together with a pipette in order to bring about a close contact. No matter how concentrated the sperm was, not a single egg was ever fertilized. As soon as the spermatozoa recovered and showed only a slight degree of motility fertilization became possible. This leaves no doubt that the motility of the sperm is one of the forces required to bring the spermatozoon into the egg.

That motility is not the only force was already indicated by the previous chapter which made it clear that even if the sperm is active it can not enter the egg unless certain physical conditions at the phase boundaries of egg, spermatozoon and surrounding solution were right. In order to leave no doubt about this fact the following experiments may be quoted. If we put NaCl sperm[14] of *purpuratus* or of *Arbacia* into a neutral mixture of NaCl + KCl containing eggs of the same species the sperm will sooner or later become very active. Yet not a single egg is fertilized. If we make the solution slightly alkaline the sperm becomes at once extremely active yet with a few exceptions no egg is fertilized; while much less active sperm will fertilize all the eggs if $CaCl_2$ is added. The second fact is this: that the most active

[14] Sperm from testicles washed in $m/2$ NaCl and kept in such a solution.

sperm of *Asterias* will not fertilize the eggs of *purpuratus* in sea water while it will do so in hyperalkaline sea water (50 c.c. sea water + 0.6 c.c. $N/10$ NaOH).

We, therefore, arrive at the conclusion that aside from the physical conditions at the surface of the egg and the spermatozoon the impact of the spermatozoon against the egg is a prerequisite for the process of fertilization.

von Dungern was, as far as the writer is aware, the first to call attention to the fact that the egg itself causes resting spermatozoa to become active,[15] but curiously enough he tried to show that only foreign sperm is "stimulated" in this way by the egg (which is, as F. Lillie pointed out, not correct) and v. Dungern tried to explain on this basis why it was not possible to fertilize the egg of the sea urchin with the sperm of the starfish which had at that time not yet been accomplished.

von Dungern noticed that the egg of the sea urchin "stimulates" the spermatozoon of starfish to greater action and he concluded that since "stimulation" according to Jennings causes "motor reaction" whereby the direction of the motile organism is changed this very stimulating influence of the egg of the sea urchin upon the spermatozoon of the starfish prohibited the latter from getting into the egg. On the basis of the same idea von Dungern was consistently led to the further conclusion that the egg exercised no "stimulating" influence upon spermatozoa of its own species and that thereby the spermatozoon of the same species was enabled to get into the egg. A year after the appearance of von Dungern's paper the writer succeeded in accomplishing the hybridization of the sea urchin egg with starfish sperm by a method which contradicted von Dungern's theory, namely, by increasing the alkalinity of the sea water whereby the spermatozoon is "stimulated" to still greater activity; and on the other hand it is a common experience that a sea urchin spermatozoon becomes more active when it comes near an egg of its own species.

The writer was anxious to compare the activating

[15] v. Dungern, *Ztsch. f. allg. Physiol.*, I, 34, 1902.

action of eggs of the same and various foreign species upon spermatozoa. Since the spermatozoa of the sea urchins are usually very active in pure sea water (*i. e.*, sea water free from egg substance) it was necessary to find a solution in which these spermatozoa will keep alive for a number of days without showing any motility. Such a solution was found in a neutral *m*/2 NaCl solution and this led to the method of putting ovaries and testes directly into such solutions instead of into sea water.[16] The ovaries and testes were first washed repeatedly in these solutions to free them from the blood or its salts, and then one drop of eggs and one or more drops of the sperm suspension were mixed in a watch glass containing 5 c.c. *m*/2 NaCl (free from egg contents). In one experiment the sperm and eggs of two sea urchins, *purpuratus* and *franciscanus*, and two starfish, *Asterias ochracea* and *Asterina* (at Pacific Grove), were used. None of the four forms of spermatozoa showed any motility in a pure NaCl solution (without egg contents). In sea water (free from egg contents) the spermatozoa of the two forms of sea urchins were very active, those of the starfish were immobile. The starfish eggs were immature and did not mature during the experiment (those of *Asterias* were out of season and very small); the sea urchin eggs were mature. The result is indicated in the following table.

That there exists no strict specificity is obvious by the fact that the immature eggs of *Asterina* activate the sperm of the sea urchin *franciscanus* as powerfully as is done by the mature eggs of the sea urchin *purpuratus* and *franciscanus*. But the spermatozoa of the two species of starfish show a marked specificity inasmuch as they are activated strongly only by the (immature) eggs of their own species and only to a slight degree by the

[16] The writer had found previously that the unfertilized eggs of *purpuratus* are killed more rapidly in sea water than in a neutral *m*/2 NaCl solution, probably on account of the greater alkalinity of the former. The same may be true for the sperm of this species, athough this has not yet been tested. The unfertilized egg of *Arbacia* is more sensitive to a pure NaCl solution than that of *purpuratus*.

TABLE I

SPECIFICITY OF ACTIVATION OF SPERM BY EGGS

| | *Asterias* ♂ | *Asterina* ♂ | *Franciscanus* ♂ | *Purpuratus* ♂ |
|---|---|---|---|---|
| *Asterias* ♀ (immature) . . | *Immediately very motile.* | No activation. | Moderately active. | Slight effect in immediate contact with egg. |
| *Asterina* ♀ (immature) . | Not motile. | *Violent activity.* | *Violent activity.* | Slight effect only near the egg. |
| *Franciscanus* ♀ (mature) | Slightly motile. | No motility. | *Immediately active.* | *Immediately motile.* |
| *Purpuratus* ♀ (mature) . | Slightly motile after some time. | Slight effect in immediate contact with eggs. | *Immediately active.* | *Immediately active.* |

eggs of the sea urchin *purpuratus*. In judging these results the reader must keep in mind first that all these experiments are made in a NaCl solution, and second, that it requires a stronger influence to activate the spermatozoa of the starfish which are at first not motile in sea water (free from egg contents) than the sea urchin spermatozoa which are from the very first very active in such sea water and which may therefore be considered as being at the threshold of activity in the pure NaCl solution.

If instead of the eggs themselves the supernatant NaCl solution from eggs is added to the sperm it is found that it requires a very much greater concentration of the supernatant NaCl solution from *Asterias* eggs to arouse the *purpuratus* sperm in NaCl into activity than if the supernatant NaCl solution from *purpuratus* or from *franciscanus* eggs is used.

The question now arises whether the relative influence of the egg on the motility of the sperm bears any relation to the power of the latter to enter the egg; or in other words if we can foretell which forms will hybridize by observing the relative activating effect of the eggs upon the spermatozoa. This does not appear to be the case on the basis of our present limited experience, since the activating effect of the *franciscanus* egg upon the sperm of *Asterias* is just as great if not greater than that of *purpuratus* eggs and yet *Asterias* sperm can enter the

latter and not the former. Even if we intensify the activity of the spermatozoon of *Asterias* by putting it in hyperalkaline sea water it will not enter the egg of *franciscanus.*

If we mix eggs of *franciscanus* and *purpuratus* in sea water and add the sperm of *purpuratus* the eggs of *purpuratus* will be fertilized more quickly than the eggs of *franciscanus;* and the reverse is true if the sperm of *franciscanus* is added to a mixture of both eggs in sea water. The writer is not quite certain that this difference is accompanied by a corresponding difference in the influence of these eggs upon the motility of their spermatozoa. It is certain, however, that the addition of egg sea water from *Asterias* does not help the fertilization of *purpuratus* eggs by *Asterias* sperm, although the egg sea water from *Asterias* increases the activity of *Asterias* sperm.

The writer is, however, of the opinion that this activating effect of the egg upon the spermatozoon is of the greatest importance for fertilization in nature and that the degree of specificity which exists (although it is far from absolute) is a means of preventing hybridization. The writer is under the impression that the eggs which are naturally fertilized in water are fertilized almost instantly after they are shed. Thus it is stated at hatcheries that the egg of the salmon loses its power of being fertilized in a few minutes and in the case of *Fundulus* the egg loses this power also very rapidly. The ripe egg of starfish dies rapidly if not fertilized. On the other hand, the writer has often been struck with the fact that the sperm of most marine forms when put into sea water is at first practically not motile. When the eggs have a specific gravity considerably greater than the water (as is the case for *Fundulus*) the eggs will sink very rapidly while the sperm remains suspended for some time. Now we have mentioned that if the absolutely inactive sperm of *Asterias* or *Asterina* comes in contact with eggs of its own species (even if they are immature) it is at once aroused into violent activity. If

the same were true for the egg of *Fundulus* fertilization could take place probably before the egg reaches the bottom of the water. If by chance a teleost of a different species would shed its sperm in the immediate neighborhood and some of it could reach the egg of *Fundulus* while it is falling the foreign sperm could probably not be aroused as quickly by the egg of *Fundulus* as the sperm of the *Fundulus* male and hence no hybridization would occur. In fish we can see that the male and female shed their sexual cells simultaneously so that they come at once in contact. The writer is inclined to believe that something similar occurs also in Echinoderms. He had last year a chance to verify once more an observation he had made for a number of years and which he had already mentioned in a previous publication.[17] The sea urchins at Pacific Grove are found in large numbers on rocks in certain coves near the shore. Up to a certain day in March every female of *purpuratus* was full of eggs. On the next day the surface of the sea in this region showed the usual indication of the spawning of large masses of animals: namely the enormous foam formation in the little coves although the sea was only moderately agitated. This foam formation is due to an increase of organic substances which lower the surface tension of the sea water and make the foam more durable. The writer realized that this might mean the end of material for some time to come and indeed not a single female of *purpuratus* of hundreds opened on that day had eggs. The condition was the same for all the sea urchins collected for two miles along the shore. During the next week immature eggs began to appear again in the sea urchins and in about ten days ripe eggs were again found. This indicates that in this region the males and females shed their eggs and sperm simultaneously. It is not impossible that among sea urchins which are found in colonies on the rocks the shedding of the sexual products of one or several individuals acts as an incentive for the whole colony. Since the eggs fall in this case also much

[17] Loeb, "The Mechanistic Conception of Life," Chicago, 1912, p. 196.

more rapidly to the bottom than the spermatozoa it is also very probable that the eggs are fertilized before they reach the bottom of the sea. We can understand under these circumstances that the specificity which exists in the activating effect of the egg upon the sperm is one of the safeguards against hybridization for eggs that are fertilized in the water, inasmuch as this specificity activates the sperm of the same species much more quickly than that of a foreign species. Other safeguards are the phase-boundary conditions which we discussed in the previous chapter.

## V

If we assume that the spermatozoon bores itself into the egg by the energy of the vibrations of its flagellum it is easy to understand the importance of its motility for this process. It is, however, equally possible that a certain energy of vibration is needed to make the spermatozoon stick to the surface of the egg and that afterwards forces of a different character bring the spermatozoon into the egg. The fact that under normal conditions a very slight degree of motility on the part of the spermatozoon allows it to enter the egg seems to favor such a view.

von Dungern had already discussed the possible rôle of phenomena of sperm agglutination in fertilization as a protective agency. F. Lillie discovered the transitory agglutination of sperm induced by a substance from eggs of the same species.[18] When the sperm of the sea urchin *Arbacia* is mixed with the supernatant sea water from eggs of the same species a cluster formation occurs which may last a number of minutes and which is essentially a transitory agglutination. In *Arbacia* the agglutination is very striking, in *purpuratus* the phenomena of agglutination are not lacking but the writer was under the impression that other phenomena of the type of tropisms might enter. But he was not very certain on this point and left that question open for further discussion. The

[18] F. Lillie, *Science*, N. S., XXXVIII, 524, 1913; *Jour. Exper. Zool.*, XVI, 523, 1914.

writer is, however, under the impression that no proof for the existence of a positive chemotropism of the sea urchin sperm for the eggs of the same species has thus far been given.

The writer observed that this phenomenon of sperm agglutination depends on the motility of the sperm:[19] It only appears when the sperm is extremely motile and it lasts only a number of minutes, often only a fraction of a minute as Lillie had found. The writer observed that the duration of the clusters depended to some extent on the alkalinity of the solution. The more alkaline the latter the more rapidly the cluster scatters. The presence of a salt with a bivalent metal, especially Ca, seems necessary for the cluster formation. Sr and Ba act like Ca and so does Mg but in the latter case a slightly higher concentration is needed. The more Ca is added the more powerful the agglutination becomes. These facts suggest the following origin of the agglutination. From the jelly surrounding the eggs a certain substance is dissolved in the sea water which reacts chemically with a certain substance at the surface of the spermatozoon. If this reaction takes place in the presence of one of the salts of a bivalent metal, especially Ca, a sticky precipitate is formed on the surface of the spermatozoa, which is slowly soluble in the solution; and the more rapidly the more alkaline the solution. If the spermatozoa are very active the impact with which they strike each other may lead to their sticking together and this agglutination will last until the precipitate is dissolved again.[20]

The writer mentions this fact here because it might give us a clue to the rôle of the motility of the spermatozoon for its entrance into the egg. One can imagine that the spermatozoon must stick to the surface of the egg in order to be taken into it and this sticking may not come about unless the spermatozoon strikes the surface of the

[19] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

[20] Lillie measures the degree of agglutination by its duration; if our assumption is correct he really measures the time required for the solution of the sticky precipitate on the surface of the spermatozoon by the sea water.

egg with a certain velocity. This is, however, merely a suggestion. The really serious difficulty of such an assumption lies in the fact that the specific and transitory cluster formation or agglutination of the spermatozoa is not a general phenomenon. It may even turn out to be confined to sea urchins and certain annelids. It is probably lacking in all cases of hybridization. Yet this would not necessarily speak against the possibility of an agglutination of the spermatozoon to the egg as a prerequisite of fertilization.

This latter idea receives some support in the writer's experiments on heterogeneous hybridization. He was able to show that both NaOH as well as $CaCl_2$, which render possible the fertilization of the eggs of certain sea urchins through the sperm of starfish, also favor the agglutination of that sperm to the chorion of the egg. This leads to the peculiar phenomenon of mere membrane formation in the egg by the living spermatozoon without the entrance of the latter into the egg.[21]

## VI

Lillie seems to take it for granted that the substance of the egg which causes sperm agglutination is identical with the substance which stimulates the spermatozoa into greater activity. If this were correct the conditions for the two phenomena should be identical, which is however far from being the case.

The writer showed that if we deprive the eggs of *purpuratus* of the jelly which surrounds them and if we wash them afterwards a few times in sea water to deprive them of the last vestiges of jelly substance which may still adhere to them they have lost completely and permanently the power of forming clusters with the sperm of their own species. Such eggs were washed four times in $m/2$ NaCl and when a drop of the supernatant NaCl solution was added to NaCl sperm of *purpuratus* which was not motile it activated the sperm very powerfully.

The writer had found that the egg sea water of *S. fran-*

[21] Loeb, *Arch. f. Entwcklngsmech.*, XL, 310, 1914.

*ciscanus* does not give a trace of agglutination with the sperm of *purpuratus* but if the experiment is made in *m*/2 NaCl solutions it can be shown that the *franciscanus* egg NaCl solution activates the NaCl sperm of *purpuratus* in an *m*/2 NaCl solution very strikingly.

The immature eggs of *Asterias ochracea* activate the otherwise non-motile sperm of the same species, but the eggs of this starfish do not give any agglutination reaction with their own sperm and Lillie found the same for the starfish in Woods Hole. It might be said that all this only proves that the activating effect requires a smaller concentration than the agglutinating effect, but may yet be caused by the same substance. This objection is, however, not tenable in the following case.

*Purpuratus* sperm washed in *m*/2 NaCl is as a rule more active in a mixture of NaCl + KCl than in a mixture of NaCl + $CaCl_2$ (if both solutions are free from egg contents); yet in the latter solution the agglutination reaction upon the addition of egg-NaCl is very strong while in the former it is lacking (unless the sperm or testicles or ovaries give off some $CaCl_2$ to the surrounding solution). Again it might be argued that the activation of the spermatozoon might be induced by the same substance as the agglutination, but that the agglutinating substance in both cases reacted with different constituents of the spermatozoon. While this may be admitted, it must also be conceded that with the facts which we have at our disposal at present we can not be certain that the aggultinating and activating substances are identical.

## VII

Lillie[22] not only takes the identity of the two substances for granted but he assumes that without the agglutinating substance in the egg (to which he gives the somewhat prejudicial name "fertilizin") no fertilization is possible. Fertilization in his opinion consists in the combination of the spermatozoon with a molecule of "fertilizin" in

[22] *Loc. cit.*

the egg, whereby the fertilizin molecule undergoes a change in the other end and this change causes the egg to develop. The fertilizin is thus an "amboceptor" in the sense of Ehrlich's side-chain theory.

The side-chain theory was invented by Ehrlich for an altogether different purpose. Bordet had found that for certain phenomena of immunity two substances were needed (which Ehrlich named amboceptor and complement, respectively). Ehrlich assumed that they were bound chemically by the antigen (the substance against which the organism was immunized) but found that while the antigen ($A$) was able to bind $B$ (the amboceptor) in the absence of $C$, it was not able to bind the complement $C$ in the absence of $B$. From this Ehrlich concluded that of the two possible modes of linkage between the three bodies $A\begin{matrix}<^{B}\\ _{C}\end{matrix}$ and $A-B-C$ the latter was the one which really occurred. Since in this case $C$ is not directly linked with $A$ but through the intermediation of $B$ he called $B$ the " amboceptor " and the scheme of linkage a " side-chain " linkage.

Lillie applies this theory (which covers the two possible modes of linkage of two chemical compounds to a third one) to the entrance of the spermatozoon into the egg, by calling the egg an antigen $A$ and the spermatozoon a complement $C$ and assuming the existence of a hypothetical amboceptor $B$ in the form of the substance that causes agglutination, the " fertilizin." Even if we are willing to overlook the fact that the egg and the spermatozoon are cells and not simple organic compounds and if we are willing to overlook the further fact that the assumption of an amboceptor as a connecting link between the two is arbitrary we can not overlook the fact that the spermatozoon does not combine chemically with the egg but that it actually enters into the egg and attaches itself to the egg nucleus. It seems then futile to discuss whether the spermatozoon combines with the egg in side-chain fashion (namely, Egg—Fertilizin—Spermatozoon) or in direct

fashion, namely,

$$\text{Egg} \begin{cases} \text{Fertilizin} \\ \text{Spermatozoon} \end{cases}$$

since the engulfing of the spermatozoon into the egg is a physical process which bears no relation to either possibility.

It has been stated that the " fertilizin theory " explains also the phenomena of artificial parthenogenesis just as well as any other theory. In a recent book on artificial parthenogenesis the writer has given the results of a large number of experiments and he has tried to explain some of them; the reader would, however, vainly look for a "theory" of artificial parthenogenesis. A theory in a scientific sense consists in the presentation in mathematical or numerical form of a phenomenon as the function of its variables. The writer has tried to prepare the ground for such a treatment of the phenomena of fertilization and of the first development of the egg by working out those variables which permit a quantitative treatment, but even if the exploration had been advanced further than it actually has been, it would not be possible to ever expect that a single theory could cover all the phenomena of fertilization and development, since under these two headings so many physically and chemically different processes are included (of which one follows the other) that they can not be covered by one theory. It is true the writer had in former publications occasionally used the term " lysin theory of fertilization " but only to express the fact that cytolytic agencies induce membrane formation and that the membrane formation induced by a spermatozoon might also be due to a cytolytic agency contained in the spermatozoon; but he has dropped this term in his recent book on the subject.

While the writer does not desire to enter into a further discussion of the side-chain theory of fertilization he wishes to point out that it rests on the claim that that substance which causes sperm agglutination is contained

in the unfertilized egg and that the egg can only be fertilized as long as this "fertilizin" is present in the egg. It is obvious that such an assumption demands for its proof that in all cases in which an egg can be fertilized it must contain the agglutinating substance. There is only one test for the presence of this substance, namely the cluster formation of the sperm in the presence of egg sea water. This proof can not be furnished since, as the writer had shown in a former paper, the reaction is lacking in many cases of hybridization; it is also lacking in the case of the starfish.[23] It is not impossible that if the theory is tested further it will be found lacking in a considerable number of cases. To this objection Lillie replies that it is not necessary that the eggs should actually give the agglutinin reaction, it is sufficient that the agglutinating substance is contained in the egg. But how can we tell that it is contained in an egg which fails to give the agglutination reaction as long as this reaction is the only reliable test for the presence of the agglutinating substance in the egg? Rigorously speaking, even if all eggs of every species gave the agglutinin reaction it would still be necessary to furnish a direct proof that the agglutinin has anything to do with fertilization and development.

It may be possible that Lillie considers such a proof to be contained in the following statement.

> I adopted then the working hypothesis that this substance[24] is necessary for fertilization and there followed immediately three corollaries, viz.: (1) if it were possible to extract this substance from eggs they would no longer be capable of fertilization; (2) fertilized eggs are incapable of uniting again with spermatozoa, hence if the hypothesis is correct they could no longer contain free fertilizin; (3) eggs in which membranes have been formed by methods of artificial parthenogenesis become incapable of fertilization; such eggs must also therefore be devoid of free fertilizin after they have reached the non-fertilizable condition if the hypothesis is correct. These consequences were actually found to be true.[25]

[23] Lillie, *Biol. Bull.*, XXVIII, 18, 1915.

[24] The "fertilizin."

[25] Lillie, *Jour. of Exper. Zool.*, XVI, 523, 1914.

Of these three "corollaries" the first one is the most important, since it claims that the power of the eggs of being fertilized varies with their contents of fertilizin. The proof consisted in this: that eggs were washed a number of times during three consecutive days and after two days the percentage of eggs that could be fertilized were diminished to about one third.

There is thus the anticipated decrease in the percentage of fertilizations.

It is a well known fact that the unfertilized eggs of the sea urchin (in fact of all marine animals) perish when they lie for some time in sea water and one of the main causes of this phenomenon is also known, namely oxidations. If the oxidations are inhibited through the removal of oxygen or the addition of KCN the life of the eggs can be prolonged.[26] In the mature starfish egg this death which is accelerated by the temperature (and has the high temperature coefficient of many life phenomena) takes place in a few hours,[27] while it begins a little later in the egg of the sea urchin. After the artificial membrane formation it takes place very rapidly also in the sea urchin egg (coincident with the enormous increase in the rate of oxidations caused by the artificial membrane formation) and in this case the death of the egg can also be retarded by the withdrawal of oxygen or the addition of cyanide.[28] In view of these facts the objection can not be avoided that in Lillie's experiment the number of eggs which could be fertilized fell off after two days to one third not on account of the loss of "fertilizin" but because of the fact that two thirds of the eggs were dead by that time. That this assumption is well grounded is testified by Lillie's own remarks:

Concomitantly, with these effects of the series of washings the developmental energy becomes greatly reduced. This was very obvious from the second fertilization.[29] On August 24 (48 hours after fertilization) a large quantity of living material was contained in the second A fertili-

[26] Loeb and Lewis, *Am. Jour. Physiol.*, VI, 305, 1902.
[27] Loeb, *Biol. Bull.*, III, 295, 1902.
[28] Loeb, "Artificial Parthenogenesis and Fertilization."
[29] Which occurred on the second day.

zation but none had even approximately pluteus structure. The most common form was a stereoblastula. In the second *B* fertilization there were a few abnormal prismatic plutei, while the majority were gastrulae. The third fertilization resulted in extremely abnormal ciliated types. The fourth and fifth did not proceed beyond abnormal cleavage stages.

From this and similar experiments Lillie draws the following conclusion:

The eggs have evidently lost something which affects their power of fertilization. Table 3 shows the measure of loss of the sperm agglutinating substance and justifies the general conclusion that this is a factor in the result. The loss of other substances may also combine in the decrease of fertilizing power, but of this we know nothing definite. As a matter of fact, fertilizing power is gradually lost with decrease of fertilizin content of the egg.

It seems to the writer that in these experiments the power of being fertilized was gradually lost by the death of the eggs. And an additional justification of this criticism is given by the following fact, that if we deprive *fresh* eggs of *purpuratus* permanently of their power of giving off "fertilizin" their power of being fertilized is not only not lost but is entirely unaltered. The writer has shown that if the eggs of *purpuratus* are treated for two or three minutes with a mixture of 50 c.c. of sea water + 3 c.c. of HCl (whereby the jelly surrounding the egg is dissolved) and if the eggs are washed they give no trace of a fertilizin reaction but 100 per cent. of the eggs can be fertilized.[30]

It might be argued that the supernatant sea water from these eggs had not lost all power of causing agglutination of the sperm. This the writer must deny but for arguments' sake he will admit that a trace near the "psychological limit" might have been overlooked where a "fertilizin" partisan might have declared that he still could perceive a faint indication of a "fertilizin" reaction. In that case only a few eggs should have been fertilized—the fertilizin theory rests on this assumption; in reality, however, practically one hundred per cent. were fertilized in every case (provided the eggs had not been lying in the see water too long, *i. e.*, more than a day or two).

[30] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

To this Lillie replies that perhaps the sperm of *purpuratus* is not so delicate an indicator for agglutinin as the sperm of *Arbacia*—but as long as the agglutination reaction is the only test for the presence of fertilizin in the egg, such an answer begs the question.

From the fact that the power of agglutinating the sperm is lost if the egg of *purpuratus* is deprived of its jelly by acid treatment the writer drew the conclusion that in this egg the "fertilizin" does not come from the unfertilized egg but only from its jelly and that this was contrary to Lillie's assumption. To this Lillie[31] replied by pointing out that the immature eggs of *Arbacia* do not give the agglutination reaction while the mature *Arbacia* egg gives the reaction very powerfully, and that we must conclude from this that the "fertilizin" contained in the jelly comes from the egg and is given off during the period of the maturation divisions (the latter statement, however, is after all only an assumption though a probable one). But this does not meet the question at issue, namely that in the egg of *purpuratus* at the time of maturity the fertilizin which is given off is contained exclusively in the jelly and not in the egg, as it should be if the presence of fertilizin in the egg were a prerequisite for its ability of being fertilized. It is true that if we repeat this experiment in the egg of *Arbacia* we find that after the removal of the jelly by HCl a trace of the agglutinating substance may still be given off by the egg, although little in comparison with that given off by the jelly. But this does not alter the facts as they are found in the egg of *purpuratus*.

As far as the two other proofs of Lillie are concerned, we have already touched upon them in the previous parts of this paper. The fact that the fertilized eggs of *Arbacia* (and of *purpuratus*) cease to give the agglutinin reaction is due to the loss of the jelly on the part of the fertilized egg to which in *Arbacia* should be added the fact that some of the material of the cortical layer is given

[31] Lillie, *Biol. Bull.*, XXVIII, 18, 1915.

off during the process of membrane formation. The writer has pointed out in former papers that the cortical layer of the egg which undergoes liquefaction in the process of membrane formation behaves towards reagents very much like the jelly which surrounds the egg.[32] But since in the egg of *purpuratus* the loss of this agglutinating power on the part of the egg is not necessarily accompanied by the loss of the power of being fertilized—*e. g.*, in the HCl experiment—we are inclined to believe that there must be another reason that an egg fertilized by sperm can not be fertilized a second time.

As far as the statement is concerned that the egg can no longer be fertilized after artificial membrane formation by butyric acid the writer can not admit the correctness of this statement (see Chapter III). In the eggs in which artificial membrane formation has been called forth by butyric acid the main if not the only block to a subsequent fertilization is the membrane itself.

This can be proved by a very simple experiment. If we call forth the membrane formation in the egg of *purpuratus* in a neutral or faintly alkaline solution of *m*/2 ($NaCl + KCl + CaCl_2$) (instead of in sea water) a very thin membrane is formed, which is easily torn and offers no resistance to the spermatozoon. All the eggs treated in this way can be fertilized by sperm. The agglutinin reaction of such eggs is, however, permanently lost.

The facts thus far known seem to force us to the conclusion that no adequate proof has been offered thus far for the connection between the power of an egg of being fertilized by sperm and its power of causing a cluster formation of the sperm. The writer has pointed out in a previous paper that it is difficult to see why there should exist such a relation, since sperm agglutination can only inhibit the entrance of the spermatozoon into the egg.

[32] "Artificial Parthenogenesis and Fertilization," Chicago, 1913, pp. 210–14. University of California publication, Physiology, Vol. 3, p. 1, 1905.

# GERM CELLS AND SOMATIC CELLS[1]

LEO LOEB

RESULTS obtained in the field of experimental pathology and especially in cancer research have an important bearing on certain problems of general biology. In the following I wish to consider connectedly some of these facts from this point of view.

I. A sharp distinction between germ cells and somatic cells has become clearly established, especially through the writings of Nussbaum and Weismann. More recent results which demonstrated that the differentiation of germ cells from the somatic cells at a very early stage of embryonic development and their non-participation in the formation of somatic tissues exists in various species, tended to emphasize this sharp distinction between somatic and germ cells.

Weismann[2] especially insisted on the radical difference between germ cells and somatic cells, inasmuch as he attributed potential immortality to the former and only a temporary existence to the latter. And Weismann regards this difference as essentially founded in the structure of both kinds of cells and fundamentally connected with the functioning of the somatic cells; this difference was obtained through selective processes as an adaptation in the struggle for existence. He does not regard the death of somatic cells as an accidental occurrence due to unfavorable conditions which it might be in our power to change, but as an inherent characteristic of somatic cells. He mentions, though casually, that the life of the cock's comb might be prolonged by grafting it on another fowl—but only to dismiss this idea as having no important theo-

[1] From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis.

[2] A. Weismann, "Ueber Leben und Tod," Jena, 1884; "Ueber die Vererbung," Jena, 1883.

retical bearing. R. Hertwig[3] also regards the death of the somatic cells as unavoidably determined by their organization which precludes necessary readjustments.

Minot[4] likewise held the life of somatic cells to be limited in duration and he ascribed this limitation to changes in cell structure, leading to a differentiation of the cytoplasm during the process of life; a change which he designated as cytomorphosis.

Within the last 14 years certain facts have been established which are contrary to this conception of a radical difference between germ and somatic cells as far as their potential immortality is concerned. Experimental investigations in tumor growth have furnished these facts. Before we state these results we have first to consider, how far tumor cells can be regarded as somatic cells. We consider here malignant tumors (cancers). They originate at various parts of the body, often under the influence of long-continued irritation. In many cases we can, if we obtain sufficiently early tumors, trace the transformation of the normal into the abnormally proliferating (tumor) tissue. This has been, as was to be expected, especially observed in the case of superficial cancers, where early stages of tumors are most likely to be encountered, for instance, in cancers of the skin, of certain mucous membranes, but also occasionally in internal cancers as in those of the stomach. In such cases the cancer cells are undoubtedly the offspring of ordinary somatic cells. There are, however, tumors, so-called teratomata, which in all probability take their origin in the germ glands and other parts of the body from parthenogenetically developing ova. But while these latter tumors do not originate from somatic, but from germ cells, the tumor cells themselves are no longer germ cells, but somatic cells in the same sense as the ordinary tissue constituents which also are derived from germ cells. We can therefore

[3] R. Hertwig, *Biol. Centralblatt*, Bd. XXXIV, No. 9, 1914.

[4] C. S. Minot, "The Problem of Age, Growth and Death," New York, 1908.

without doubt regard tumor cells as a kind of somatic cells.

One of the most characteristic properties of cancer cells is their ability to grow after transplantation into other animals of the same species. This applies not to all, but to a certain number of spontaneous cancers; the majority of spontaneous tumors are not transplantable into other individuals of the same species. They grow, however, usually after transplantation into the same individual in which they originated. There does not exist as far as their origin is concerned any essential difference between these two kinds of cancers—those transplantable and not transplantable into other individuals. The cancers used in experimental tumor investigation take their origin from somatic cells; but it appears some are less sensitive to the difference in the chemical composition of the body fluids which exists between different individuals of the same species than others, and those less sensitive can be transplanted, while others can not.

In those tumors which are transplantable, relatively few tumor cells give after inoculation into other animals origin to the new tumors, and the tumor cells after the first transplantation not rarely multiply with greater vigor than they did in the original animal, an effect caused, as I could show, through the stimulating influence of the cutting and otherwise manipulating the tumor cells. In each animal therefore there are produced many successive generations of tumor cells, and after transplantation into another individual each surviving cancer cell produces again new generations. Consecutive transplantations into many individuals have been carried out with the same tumor. The potential proliferative power of the cancer cells is therefore enormous. It is, however, not so much the intensity of the proliferative power of the tumor cells which we wish to consider as the potential duration of their life. It has been shown that epithelial, as well as connective tissue tumors can be transplanted through many generations and can survive for a long time the animal in which the tumor originated. Thus I was able

to transplant the connective tissue cells of a rat sarcoma through forty successive generations of animals, and it was merely the result of accidental bacterial infection due to the unfavorable conditions under which the work had to be carried out which caused the ultimate death of the propagated cells.

An epithelial tumor found by Jensen in a mouse has been propagated in various laboratories through a period of almost fifteen years, and another epithelial tumor of the mouse we have been propagating in mice for a period of seven or eight years, without any sign of diminishing vitality in the propagated cells being noticeable.

In all these transplantations of tumor cells, be they of connective tissue or epithelial origin, it could be shown that the peripheral cells remain alive and from these surviving cells the cell growth starts. These observations suggested to me in 1901 the conclusion that tumor cells may have a potential immortality in a similar manner as germ cells,[5] and inasmuch as tumor cells are only modified somatic cells, I furthermore concluded that the same statement holds good in the case of somatic cells.[5] Further experiences in the field of experimental tumor investigation during the following years confirmed this conclusion and permitted its enunciation with greater definiteness.[6] The potential immortality of the somatic cells of course can only be made probable, it can never be definitely proved, inasmuch as our experience merely deals with finite periods. But the same restriction holds good in the case of the germ cells in which the potential immortality is likewise merely a strong probability and not a definitely proven fact.

Weismann believed that protozoa are in the same sense potentially immortal as germ cells, in contradistinction to somatic cells which do not possess potential immortality. Some facts were, however, discovered which, according to

[5] "On the Transplantation of Tumors," *Jour. Medical Research*, Vol. VI, No. 1, 1901, p. 28; *Virchow's Archiv*, Bd. 167, 1902, p. 175.

[6] "Tumor Growth and Tissue Growth," *Am. Philosophical Society*, XLVII, 1908, and at other places.

the interpretation given them, seemed to contradict Weismann's conception. Thus Maupas found that various kinds of infusoria did not propagate by fission indefinitely, but that a sexual process, conjugation, was necessary at certain times, and Calkins showed that there were regular periods of depression, and while a spontaneous recuperation from the effects of certain depressions could take place and in still other cases artificial stimulation would aid the animals in overcoming the critical periods, at other times depressions proved fatal without an intervening conjugation. Woodruff, however, by choosing conditions of environment more in accordance with the conditions found in nature, could keep a strain of *Paramæcium* apparently indefinitely alive without any intervening periods of copulation being required. This seemed to point to a potential immortality of protozoa in the sense of Weismann. Recently, however, Woodruff and Erdmann[7] found that the recovery from depression which takes place is accomplished through nuclear changes comparable to, but not identical with, those observed during copulation. This seems in some respects to agree with R. Hertwig's previously enunciated theory according to which depressions and senility in cells are due to a disproportion between the nuclear and cytoplasmic material, and that recovery from such unfavorable conditions depends upon the reorganization of the nucleus, essentially consisting in a diminution of the mass of the latter. Inasmuch as in metazoa—he concluded, further—such a rearrangement between nuclear and cytoplasmic masses can only take place in the case of germ cells, but not somatic cells, only germ cells are immortal, while somatic cells are necessarily mortal. While Weismann regarded the unavoidable mortality of the somatic cells as a secondary acquisition, the result of a process of selection, the death of somatic cells being of advantage to the propagation of the race, Richard Hertwig[8] regards the death of somatic cells as inherent in their structure, which precludes the

[7] *Biol. Centralblatt*, Bd. 34, August, 1914, p. 484.
[8] *Biol. Centralblatt*, Bd. XXXIV, 1914, No. 9.

possibility of nuclear reorganization of the cell necessary for continued life. In a somewhat related way, Minot considers, as mentioned above, the death of somatic cells as inevitable and as the result of cytomorphosis, which means the relative increase in size and differentiation of somatic cells during life. In this connection it is interesting to note that while R. Hertwig considers (in protozoa primarily, but secondarily also in other cells) an increase in the size of the nucleus—the result of the activity of the cells—as the cause of functional disturbances leading to senility, Minot on the other hand connects senility with a relative decrease in the size of the nucleus and an increase in the mass of the cytoplasm. Now as far as the protozoa are concerned, the controversy does not seem to concern so much the potential immortality of these organisms as the problem as to whether the individual protozoon corresponds to a germ cell or to a somatic cell of a metazoon, or whether it partakes of the character of both. There can be little doubt that individual protozoa possess potential immortality, a conclusion which would not be invalidated through a loss of certain parts of the protozoon body at certain periods of its life cycle.

*We may therefore conclude that all three kinds of cells, protozoa, germ cells, as well as certain somatic cells of metazoa, possess a potential immortality.*

Tumor cells are somatic cells in which such secondary changes leading to a cessation of proliferation as take place under certain conditions in all the individual cells in some kinds of somatic tissues, are affecting only a certain number of cells. In the case of some somatic cells, as, for instance, those of the epidermis, it is evident that the secondary changes in structure and metabolism, which lead to a cessation of proliferative power, are due to unfavorable conditions of blood-supply. What Minot calls cytomorphosis can therefore in this case be referred not to necessary transformations inherent in the cells, but to unfavorable environmental conditions into which the cells are placed as a result of their multiplication. Such secondary degenerative changes take place also in tumor

cells under similar defective conditions of blood-supply. Here also degenerative changes entail a cessation of proliferation in a similar manner as in ordinary tissue cells. While farther away from the blood-vessels the tumor cells degenerate and die, near the blood-vessels they continue to live and to multiply.

While from a theoretical point of view, therefore, the question as to the potential immortality of somatic cells has through the experiments on tumor cells been answered in a decisive manner, it was nevertheless of interest to extend these investigations to ordinary tissues. Such investigations we undertook in the course of the last eight years, and while certain obstacles were encountered, which prevented the continued life of ordinary tissues, the results were of interest in giving an insight into some of the conditions which determine the growth, life and death of somatic cells. These investigations have shown that if tissues are transplanted into another individual of the same species, under the influence of the constitution of the body fluids, which differs in different individuals of the same species, the metabolism in the transplanted tissues is interfered with as shown, for instance, in pathological differences in pigmentation seen in black skin of the guinea-pig after transplantation into other animals of the same species. After transplantation of pigmented skin into the same individual in which it originated, such pathological changes do not occur. As I have previously pointed out, a certain adaptation exists between the tissues and body fluids in animals of the same species, and even between the tissues and body fluids of the same individual. Thus it comes about that the interaction of tissues and body fluids of the same species leads to different and less toxic products than those produced through the interaction of the body fluids of one with the tissues of another species. Even the interaction of tissues of one animal with the body fluids of another animal of the same species leads to more toxic products than the interaction of body fluids and tissues of the same individual. In the latter case toxic products, interfering with the life of

normal tissues, are not produced, while in the former cases such products acting directly or indirectly are formed. As an illustration of such a specific relationship between body fluids and tissues, I cited the specifically adapted effect which tissue coagulins exert on the constituents of blood plasma.[9]

Now as the result of these differences in metabolism induced through the differently constituted body fluids the lymphocytes begin to invade the transplanted tissues, and the invading connective tissue does not preserve, as it does after auto-transplantation, its young and cellular state, but produces fibrous bands which contract around the parenchyma after homoiotransplantation, and thus exert pressure. Both connective tissue and lymphocytes destroy thus the homoiotransplanted tissue, while they usually spare the autotransplanted tissue the metabolism of which is normal. In the case of certain tissues, as, for instance, kidney, however, even after autotransplantation into the subcutaneous tissue the metabolism of the transplanted cells becomes abnormal under the abnormal conditions under which they now live, and here the lymphocytes and connective tissue destroy, therefore, even the autotransplanted tissue, although at a later date than the homoiotransplanted kidney tissue.

The fitness of a tissue in an individual determining its power to live or to grow depends, therefore, on two factors: (1) on the specific adaptation existing between tissues and body fluids, and (2) on the way in which various substances are carried to the tissue. A perfect nutrition implies the carrying of the food substances to the tissues in the normal way through blood-vessels. It is probable that on the intact relations between capillary endothelium and parenchyma cells depends such a sifting of various food substances and waste products as is best suited to the normal metabolism of the cells. If, as in the case of the kidney tissue, this mechanism is disturbed, abnormal substances are produced notwithstanding the specific adaptation existing in this case between tissue cells and body

[9] "Immunity and Adaptation," *Biol. Bulletin*, Vol. IX, 1905, p. 141.

fluids after auto-transplantation. And it seems that a perfect fulfilment of the second requirement might even be able to overcome a deficiency in the first condition, the specific adaptation between tissues and body fluids. This seems at least to be the case, whenever a kidney is successfully transplanted into another individual of the same species and lives here for a long period of time.

A peculiar resistance to foreign body fluids is apparently shown by the germ cells. They represent in reality individuals residing in a host organism of the same species. In this case the host organism is nearly related to but not identical with the individuality of the germ cells. In some respects we have, therefore, here a condition comparable to one existing after homoiotransplantation of tissues. And still the germ cells do not show any signs of injury. There is, therefore, in germ cells as yet lacking that substance which has a specific affinity to certain parts of the body fluids, or through their situation the germ cells are somehow protected against the injurious influence of these substances.

Thus it comes about that through transplantation into other individuals of the same species the potential immortality of the ordinary tissues can not be demonstrated. This applies to the tissues investigated so far.[10] However, it is quite possible that we may yet find that in the case of certain tissues the life may be permanent even after homoiotransplantation. It was furthermore thinkable that through serial transplantation, retransplanting the tissue at an early date before the lymphocytes and connective tissue had had a chance to seriously injure it, better success could be obtained. In the case of the skin I have undertaken such serial transplantations some years ago; our investigations have, however, in this case shown that it was not possible to retransplant this particular

[10] Leo Loeb u. W. A. F. Addison, *Arch. f. Entwicklungsmechanik*, Bd. XXVII, 1909, p. 73; Bd. XXXII, 1911, p. 44. Max W. Myer, Bd. XXXVIII, p. 1, 1913. Llewellyn Sale, Bd. XXXVII, 1913, p. 248. M. G. Seelig, Bd. XXXVII, 1913, p. 259. Cora Hesselberg, *Journ. Experimental Medicine*, Vol. XXI, 1915, p. 164.

tissue indefinitely;[11] these experiments ought to be extended; especially might it be of interest to use the direct descendants as hosts for the tissues of the parent. It is to be expected that the quality of the parents which makes the body fluids suitable for their own tissues might make them likewise suitable for certain of their offspring. Such experiments I began some time ago and I expect to continue them if opportunity should present itself.

The growing of tissues in culture media, which excludes attack on the cells by connective tissue and lymphocytes, may also serve the same purpose and quite recently has been used through a larger number of generations. But, as stated above, we have in these experiments merely to deal with an attempt to confirm the potential immortality of the somatic cells which had in principle been established through previous investigations on the life of tumor cells.

While thus various kinds of tissues of an organism have the potentiality of an immortal life, separated from the organisms to which they belonged, the organism as a whole invariably dies and with it its component tissues. This is evidently due to the interdependence of various parts of an organism and to the death of certain sensitive cells, especially the ganglia cells of the central nervous system. We might therefore be inclined to conclude that these ganglia cells do not possess the potentiality of immortal life. But even in the case of the ganglia cells, which are of such significance for the life of the organism as a whole, we can at present not deny the possibility that they also may have the potentiality of immortality and that they merely succumb under the influences of certain injurious conditions arising in the organism. On the other hand, fully developed ganglia cells have apparently lost the power to multiply; they are furthermore sensitive to certain insults to which other tissues show resistance. Thus the unfavorable condition prevailing during the process of transplantation into another organism and directly afterwards seems to be sufficient to cause the death

[11] Leo Loeb, *Archiv. f. Entwicklgsmch.*, Bd. XXIV, 1907, p. 638.

of certain ganglia cells when other tissues would survive. But neither of these facts prove that under favorable conditions very much differentiated cells, like ganglia cells, might not have the power to live indefinitely, although they have lost the power to multiply. Sensitized connective tissue cells of the uterus may after transplantation into the same or another individual in which corpus luteum substance is circulating grow very energetically and produce placentomata, while after transplantation of fully developed deciducomata no further growth can be obtained and all or almost all the cells die. Here also the fully "differentiated" cells have lost the power to multiply and at the same time they have apparently become more sensitive to the effect of injurious influences than young and yet undifferentiated predecidual cells of the uterine mucosa.

But here we can observe that some strands of fully differentiated placentoma tissue may survive even under those unfavorable conditions—without, however, resuming growth or returning to the undifferentiated condition—namely, such strands of tissue as are situated under the best environmental conditions, in close proximity to the host tissue, at places most accessible to the foodstuffs or oxygen supplied by the circulating blood or by the peritoneal fluid. This suggests that even much differentiated cells which have lost their power to propagate may still have the power to live, when kept under favorable conditions, and that their death is the result of unfavorable environmental influences. Thus we must at least admit at the present time the possibility that also the ganglia cells, while they do no longer multiply, may still possess a potential immortality; that cellular differentiation precludes the latter possibility has as yet not been demonstrated. We must therefore sharply distinguish between the power of cells to grow and their power to live; while the former seems to be destroyed through differentiation —at least in some cases—the latter may still exist. In the case of other tissue cells we have it to a certain extent in our power through experimental conditions

to prevent those changes which lead to differentiation and death; thus in the case of tumor cells through constant transfer into a new host we can enormously increase the number of as yet less differentiated cells on which the propagation depends by causing an intense multiplication of these tumor cells, many of which, if left in the same organism, would have undergone secondary degenerative changes. Through experimental means, viz., through the transplantation into different kinds of hosts the relative preponderance of propagation and differentiation of tumor cells can be varied. We furthermore know that through "chemical" sensitization combined with mechanical stimulation, the same effect can be produced, at least temporarily, in the connective tissue cells of the uterine mucosa. Under those conditions a large number of cells are induced to propagate and remain young while their offspring gradually change into fully differentiated cells. If we grant the possibility that differentiation of such cells as ganglia cells, while it entails loss of the power to propagate and greater sensitiveness to insults, does not necessarily mean the necessity to die, then the problem of prolongation of life would to a great extent depend upon the possibility of preventing injurious influences which at present disturb the function of ganglia cells from attacking these cells and causing their death.

II. The germ cells are potentially immortal, but this potential immortality can only be realized, if at certain periods certain changes take place within the cells, which concern especially the nucleus, phenomena consisting in maturation, followed by fertilization or parthenogenetic development. In a similar manner the observations of Woodruff and Erdmann suggest that at the time of depressions in the life of the protozoa, possibly similar nuclear phenomena take place at least in certain cases. We have seen that in the case of somatic cells there are also indications of the existence of potential immortality. The question may therefore be raised, whether similar periodic rearrangements of the nucleus, as in the case of the germ

cells and protozoa, may not also take place in the case of the somatic, especially of tumor cells. Without considering any connection with the problem of the immortality of the somatic cells, Bashford, Murray and Bowen[12] stated on an empirical basis that in charting the number of successful inoculations of a mouse carcinoma in mice, in different generations and in different strains of the same generation, they noticed definite rhythmic variations in the number of successful inoculations, a maximum of successful inoculations in one generation being followed by a minimum in the succeeding generation. However, they also state that parallel strains of the same tumor did not show maxima or minima at the same time. Bashford, Murray and Bowen, in order to explain these observations, assumed that different parts of the same tumor show different degrees of growth energy at the same time; this would imply that such areas differing in growth energy at the same period are separated through transplantation; so that in one generation mainly energetically growing pieces are used for transplantation in the succeeding weakly growing pieces almost altogether, an assumption which does not appear very probable.

Calkins[13] held that there occur in succeeding generations not so much rhythmic variations in the number of successful transplantations as in the growth energy of the tumors. He compared these rhythmic variations with the rhythms observed by him in the case of *Paramœcium*. However, such rhythms were not noticeable in the mouse carcinoma which we have propagated for a number of years in our laboratory, as has been shown by Moyer S. Fleisher.[14] He furthermore shows that even in the case of those tumors, on the study of which Bashford and his collaborators and Calkins base their conclusion, it is very probable that the variations which these authors observed do not represent definite rhythms, but are, as far

[12] Bashford, Murray and Bowen, *Zeitsch. f. Krebsforschung*, 1907, Bd. 5, Heft 3.

[13] Calkins, *Jour. Exper. Med.*, 1908, X, 283.

[14] Moyer S. Fleischer, *Zeitschrift f. Krebsforschung*, Bd. 14, Heft 1, 1914.

as their conclusions are not based on methods of determining the growth energy of tumors, not suitable for this purpose, in all probability merely the expression of the existence of a number of uncontrolled variable factors. Such factors are numerous and they may explain certain variations observed in growth energy and number of takes in transplantations undertaken at different times or in different mice. There exists, therefore, at the present time no evidence making even probable the existence of rhythms of growth and vitality in somatic cells comparable to those found in protozoa; neither have thus far been found in somatic cells indications of nuclear changes similar to those periodically occurring in germ cells and probably also in some protozoa and apparently bearing some relation to variations in growth and vitality in these cells. At present we must therefore reckon at least with the possibility that the immortality in somatic cells is not connected with rhythms in vitality and in nuclear changes of such a character as observed in the other two kinds of potentially immortal cells.

III. External factors acting on an organism may exert an influence on its germ cells and here produce certain changes which may be transmitted to the following generations, and thus through a number of generations the offspring may show deviations from the type, although the character of the lesions appearing in different generations may not be identical. This has been observed as a result of the action of poisons such as lead and alcohol. Especially the extensive investigations of Stockard on the action of alcohol in guinea pigs demonstrate conclusively that defects appear through several generations. In the case of these injuries transferred to the offspring, it is doubtful whether and to what extent these inheritable defects are characteristic for a certain poison, or whether we have to deal with traumatisms which might be caused in a similar manner by many poisons or even by injurious physical agencies. There is some evidence tending to show that most diverse chemicals may influence embryonic development in a similar manner, that they may produce

identical defects in the developing organisms. It seems to be otherwise in the case of certain external conditions which produce first changes in some somatic cells which on their part apparently induce such secondary changes in the germ cells that in the offspring, not exposed to the external conditions that affected the parents, again changes appear in some somatic cells similar to those produced in the parents through the external conditions. Such results were published by Kammerer. In the latter case the changes produced and transmitted to the offspring showed evidently a characteristic specific relationship to definite external conditions. Such a specific relationship is, as far as we can judge at present, lacking in the case of defects or deficiencies produced through the action of poisons.

I wish to report briefly on a change which my collaborators Moyer S. Fleisher, Miguel Vera and myself[15] have produced in somatic cells, a change which is transferab'e to the following cell generations and is therefore hereditary, and which, while it would be pronounced non-specific, if we should use ordinary criteria, can through the use of special methods be shown to possess a definite, characteristic relationship to the external factor that caused this change and must therefore be called specific. The observations on which these conclusions are based are briefly as follows:

If we inoculate mice with the mouse carcinoma used by us in our experiments and from the ninth to the fourteenth day after inoculation give on successive days four intravenous injections of such substances as colloidal copper or hirudin, a marked inhibition in growth takes place during the period of injection. The intensity of this inhibition varies in different cases and it is possible for us by using a combination of two substances to cause a retrogression of a considerable number of tumors. Now,

[15] Moyer S. Fleisher and Leo Loeb, *Jour. Exper. Med.*, Vol. XX, 1914, p. 503. Moyer S. Fleisher, Miguel Vera and Leo Loeb, *Jour. Exper. Med.*, Vol. XX, 1914, p. 522. Moyer S. Fleisher and Leo Loeb, *Jour. Exper. Med.*, Vol. XXI, 1915, p. 155.

if we inject daily from the second to the fifth day after inoculation mice with either of these two substances, tumor growth is not noticeably retarded through the injections. Tumors, in an early stage of development, are resistant to this inhibiting effect. If we now give four intravenous injections, one on each successive day, from the second to the fifth day, and later again to the same mice four injections from the ninth to the thirteenth day, the latter series of injections which had been effective in other animals not previously injected from the second to the fifth day, have now almost completely lost their efficacy. The mice have through the first set of injections become immune against the action of colloidal copper and hirudin as far as the effect of these substances on tumor growth is concerned.

We next inquired into the mechanism of this immunity, and especially were we concerned with the place where this immunity is produced. It was conceivable that this took place either in some organ of the injected animal or in the tumor cells themselves. The following experiments showed that both possibilities were realized: If we inject the mice on the four days preceding inoculation with tumor, immunity is produced, at least in the case of colloidal copper. This proves that some organ in the host animal contributes to the immunity, inasmuch as in this case the preliminary injections exerted their influence without having had a chance to act on the tumor cells. But the tumor cells themselves also become actively immune, as shown in the following manner: We inject animals with either colloidal copper or hirudin from the second to fifth day, and again from the ninth to the thirteenth day after inoculation. Two days after the last injection we used the tumors of some of the injected animals for reinoculation into a new set of mice. Nine to thirteen days after this second inoculation the mice belonging to the second set are injected with colloidal copper and hirudin, respectively. Now we find that these tumors also are almost entirely resistant against the effect of colloidal copper and hirudin, although in this case no preliminary injections had been given to the animals which

are now the bearers of the tumors. It is therefore necessary to conclude that the tumor cells used for transplantation are in this case the sole bearers of the immunity, and that the tumor cells themselves have been actively immunized. From the latter experiments we may furthermore conclude that the immunity acquired by tumor cells is transferred to the following generations of tumor cells, and that therefore a hereditary transmission of a character acquired by somatic cells under the influence of external conditions takes place. The conclusion is based on the following consideration: The process of tumor inoculation consists in the transfer of a very small particle of tumor. Very soon after transplantation most of the transplanted tumor cells become necrotic and only a relatively small number of peripheral tumor cells remain alive. These very soon begin to proliferate, and through their proliferation give origin to the developing tumor. If therefore the tumors developing after transplantation in the new hosts are immune, the immunity must have been transmitted from the few cells remaining alive after inoculation to the new cell generations to whom they give origin. A fully developed tumor represents a combination of a large number of generations of tumor cells and it may be assumed that the later generations of tumor cells preponderate numerically very much over the earlier generations. Through how many generations of tumor cells this transmission of the acquired immunity can be propagated remains yet to be determined. From our preliminary experiments, which are, however, not yet definite, it appears not improbable that it extends at least through several series of transplantations.

Both colloidal copper and hirudin inhibit tumor growth. The sign by which we judge the effect of these substances is therefore essentially the same in both. We might thus be inclined to conclude that their action is identical and that likewise the immunity which they produce is the same; that animals having received preliminary injections of colloidal copper would therefore be immune not only against the action of colloidal copper but also against

hirudin, and that those having received preliminary injections of hirudin would also be immune against colloidal copper. We wished to test this conclusion and undertook therefore experiments in which we immunized animals with one substance and examined later their immunity not only against the substance with which they were immunized, but also against the other substance. These experiments showed that animals immunized with colloidal copper are essentially only immune against the effect of colloidal copper, not of hirudin, and those immunized with hirudin are immune against hirudin, but not noticeably (very weakly, if at all) against colloidal copper. The acquired immunity is therefore a specific one. This specificity can be shown to exist if after preliminary injections given from the second to the fifth day after inoculation the immunity is tested through cross injections given from the ninth to the thirteenth day. It can also be demonstrated in the tumors transplanted into other animals after preliminary injections in the first set of animals. The specificity concerns therefore the immunity which is produced in the tumor cells and probably also the immunity in the organism of the injected animals. These investigations prove then (1) that an acquired immunity against the injurious action of certain substances can be localized in the cells concerned; (2) that this immunity can be transferred to later cell generations; and (3) that although the effect of two substances on the cells is apparently the same, the mechanism through which this effect is produced differs in the case of each substance, and that therefore the immunity produced against the injurious action of these substances is a specific one for the substances injected. We see therefore that in a similar manner as in germ cells an effect produced through an external agency can be transmitted to later generations; a transmission of changes produced through an external (chemical) agency may be transmitted to later generations also in the case of somatic cells. But the further results we obtained in the case of somatic cells suggest the question whether the lesion produced and transmitted

in germ cells may not also be specific, although different chemicals produce apparently the same results. May there not in germ cells, just as in somatic cells, exist a difference in the mode of production of these lesions through the different substances and consequently a specificity in the acquired lesion, notwithstanding the apparently unspecific character of the lesion? Our work makes it possible that this question which seems of considerable theoretical interest may be solved in a similar manner as in the case of the somatic cells, viz., through testing the immunity produced through the action of chemical substances.

## SUMMARY

1. It is shown that evidence similar to that which makes probable the potential immortality of protozoa and germ cells also exists in the case of somatic cells of metazoa. As far as the protozoa are concerned, the discussion does not, as seems to be assumed, concern their potential immortality so much as the question whether protozoa correspond to germ or to somatic cells of metazoa or represent perhaps a combination of both.

2. While in the case of tumor cells the potential immortality of somatic cells has been demonstrated as definitely as the character of the problem will ever permit, the difficulties standing in the way of a similar demonstration in the case of certain other somatic cells and the means of overcoming these difficulties are analyzed. It is particularly shown that chemical differences existing between the body fluids of individuals belonging to the same species are the basis of these difficulties, and that as a result of these differences the metabolism of cells in a new environment is modified in such a way that the behavior of connective tissue cells and of lymphocytes is altered, and that as a result of these alterations the death of the tissue is brought about where it could probably have lived indefinitely. Besides altering the character of the body fluids, transplantation of tissues furthermore has usually an additional injurious effect; it changes the way in which nourishment is carried to the transplanted cells, and this

may also lead to alterations in metabolism calling forth a destructive activity of connective tissue and lymphocytes. Thus there exist difficulties in the case of certain tissues in demonstrating through serial transplantation their potential immortality, in a similar manner as it has been demonstrated in the case of strongly proliferating somatic cells (tumor cells).

It is also shown that in the case of the germ cells which really represent a foreign individual within a host organism mechanisms exist which prevent these injurious agencies from becoming effective.

3. We must sharply distinguish between the power of cells to grow and their power to live. While the former seems to be destroyed through differentiation, the latter may still exist, and we can therefore at present not deny the possibility that even highly differentiated somatic cells may still possess the potentiality of immortal life.

4. While in the case of protozoa and germ cells definite cycles exist, manifesting themselves either through the occurrence of rhythmically occurring depressions of vitality or of typical changes in the nuclei, such cycles have so far not been demonstrated in the case of somatic cells, particularly of tumor cells.

5. In the case of germ cells external factors can produce certain changes, and these changes (not necessarily identical with those originally produced through the external factors) can be transmitted to the offspring. It is shown that in a similar way in the case of somatic (tumor) cells a transmission of characters acquired under the influence of external agencies to the succeeding cell generations may take place. It can be shown in the case of the somatic cells that apparently similar changes produced through different external agencies are really not identical, but specific. It is suggested that such a specificity of transmitted characters may also exist in the case of germ cells despite the apparent identity of changes produced through different external agencies.

# MENDELIAN INHERITANCE OF FECUNDITY IN THE DOMESTIC FOWL, AND AVERAGE FLOCK PRODUCTION [1]

DR. RAYMOND PEARL

IN 1912 I showed,[2] from extensive experimental data that, in certain breeds of domestic poultry, winter egg producing ability is inherited in a strictly Mendelian manner. It was pointed out that there was much evidence indicating that winter production was, on the whole, a rather reliable index of total fecundity capacity. As was to be expected, the novelty of the results presented in the papers referred to led to their criticism from various points of view, including that of the practical poultryman. Most of these criticisms have been based upon some misunderstanding of the nature of the results themselves. Others, and particularly those of the poultry press, have apparently been based on a purely conservative instinct to resist the intrusion of any new idea which seems to threaten those solid personal and editorial assets of (reputed) infallibility and "safe and sane" judgment.

It has seemed to the writer more likely to conduce to the advancement of knowledge in this field if he went steadily about collecting more and more concrete objective evidence rather than engaging in polemic disputations with everyone whose opinion in regard to the validity or interpretation of the earlier results chanced to differ from his own. As a result of this policy there has accumulated a large mass of additional experimental data confirming and extending the results of the earlier work.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 81.

[2] Pearl, R., "The Mode of Inheritance of Fecundity in the Domestic Fowl," *Jour. Exper. Zool.*, Vol. 13, pp. 153–268, 1912. *Cf.* also "The Mendelian Inheritance of Fecundity in the Domestic Fowl," AMER. NAT., Vol. XLVI, pp. 697–711, 1912.

This material will be published as opportunity offers.

It is the purpose of the present paper to record certain facts which are pertinent to a general consideration of the problem of inheritance of fecundity, but at the same time do not fall in the direct line of the experimental inquiry. They are matters, in other words, which are essentially by-products of the investigation but still have a more or less important bearing on the interpretation, in a broad sense, of the whole.

## I. The Seasonal Distribution of a Flock Egg Production under a Mendelian System of Breeding as compared with Simple Mass Selection

The mean egg production per bird in the different months of the laying year has been given by Pearl and Surface[3] in an earlier paper. Those results are based on the weighted mean production of the flocks of Barred Plymouth Rocks at the Maine Agricultural Experiment Station during the ten years that a system of mass-selection was followed in breeding for egg production.

It is an obvious deduction from the results of the Mendelian experiments recorded in the earlier papers already referred to, that by their application it should be possible to modify the average production of a flock over a rather wide range, the modification being of a fixed and permanent character under any definite conditions of environment and breeding. To many practical poultrymen the only test of the validity of the conclusions reached which has any significance, is that of average flock production. It is obvious that from a technically critical point of view such a test has, of itself, relatively small value in helping to judge of the correctness of a Mendelian interpretation. At the same time it is clear that if one takes a flock of poultry of mixed genetic constitution in respect of fecundity and aims to preserve in his breeding only animals carrying both the factors $L_1$ and $L_2$ necessary for high

[3] Pearl, R., and Surface, F. M., "A Biometrical Study of Egg Production in the Domestic Fowl." II. Seasonal Distribution of Egg Production," U. S. Dept. of Agr., B. A. I. Bull. 110, Pt. II, pp. 81–170, 1911.

production, there ought to result a marked and immediate improvement in average flock production no matter what the size of the flock.

This, as a matter of fact, is exactly what has been done in the breeding of the flock of Barred Plymouth Rocks at the Maine Station for several years past. No attempt has been made to propagate low fecundity strains, after it had once been demonstrated that this could be done. In the work since 1912 the experimental aims have been such as not to be at variance with the practical one of getting the most eggs with the least trouble and expense, so far as has concerned the Barred Plymouth Rock stock. Consequently in making the matings from which the foundation Barred Plymouth Rock stock was being maintained I have each year endeavored to keep a number of different blood lines comparatively pure for the factors $L_1$ and $L_2$, and then intercross these lines with one another.

The results have been highly successful from a practical point of view. This is indicated by the figures shown in Table I and graphically in Fig. 1. These compare the mean egg production per bird month by month under the old system of mass-selection and under the new system of breeding which recognizes the Mendelian inheritance of fecundity with sex-linkage of the factor on which high production depends. The figures for the new system are those of the laying year 1913–14. In the laying year 1912–13 the flock had not yet attained any considerable degree of homogeneity in respect of fecundity factors since up to and including the preceding year low producing genetic combinations had been deliberately propagated and therefore an average which included all birds in the flock would be manifestly unfair as a test of the practical worth on a large scale of the new systems of breeding. The laying year 1913–14 is then the first completed year on which records are available for a fair test of the Mendelian plan on a total flock scale.

The Barred Rock flock of the year 1913–14 included 192 birds which completed the year's work. A number of other birds (about 20) began the year but died before its

completion. These 192 birds were divided among three flocks of 125 each, the other birds in each flock being cross-breds of various sorts.

It is possible to compare these 1913–14 flock with the old records during nine months of the year only. The reason for this is found in the fact that the trap-nesting season is, under the present system of management, brought to a close with August. Furthermore a record is now kept of the laying of the pullets in October at the beginning of the year, whereas formerly the season's records did not begin until November 1. This comparison is made in Table I. Also in this table the production for 1913–14 is compared with the *best* single year during the mass selection experiment, when anything approaching a corresponding number of birds were included,[4] and for which all environmental conditions may be regarded as approximately normal.[5] The single year records which come nearest to fulfilling all the conditions for a fair comparison with 1913–14 are those for the 100-bird pens in the laying year 1905–06. There were two such pens and 182 birds survived through the year. There was one small environmental accident in that year which reduced the production in May somewhat.[6] There were adverse environmental influences in 1913 probably quite as effective in reducing production as anything that operated in 1905–06. The seasonal conditions, size of flock, etc., were all fairly closely comparable with those obtaining in 1913–14. At that time (1905–06) the flock had been under continuous mass selection for eight years.

There are a number of difficulties in the way of making a comparison between any single year now, and the "best

[4] The absolutely best single year under mass selection was 1901–02. That year there were only 48 birds for which records are available. These were in several respects a special lot, and can not fairly be compared with large flocks kept under ordinary flock conditions. Cf. Pearl and Surface, *loc cit.*

[5] *Cf.* Pearl, R., and Surface, F. M., "A Biometrical Study of Egg Production in the Domestic Fowl. I. Variation in Annual Egg Production," U. S. Dept. Agr., B. A. I. Bull. 110, Pt. I, pp. 1–80, 1909, for an account of the environmental difficulties in certain of the earlier years.

[6] See Pearl and Surface, *loc. cit.*, p. 18.

year'' made under the mass-selection system. In the first place in order to make the comparison at all fair the flocks in which the birds were kept when the records were made must be of approximately the same size. It has been conclusively demonstrated by earlier work in the laboratory that egg production becomes reduced as flock size increases. It would be idle to compare the results now where the birds run in flocks of 125 to 150 birds per pen with the ''best'' of those prior to 1904, when the flocks were never larger than 50 birds each and were sometimes smaller. This restricts single year comparisons then to the period after 1904.

In the second place, if we take the year when the total production was highest, as the ''best'' year, we shall find, in practically every case, that some particular month or months of this year will fall below the average for that month or months. There are then two alternatives, either, on the one hand, to take for comparison with a single year now that year under the old system of breeding which, on the whole, is the best and then make allowances for disturbing factors in particular months, or, on the other hand, to compare a single year now, month by month, with an artificial year's record made up by picking out the best record of each individual month regardless of the year in which it occurred or of the size of flock. The second of these comparisons is obviously artificial, since it is continued high production month after month in the *same* laying year which is important. It is of interest, however, to see the results of the comparison on both bases. These comparisons are made in Table I.

The best single year 100-bird pen record in the earlier period is, as already pointed out, that for 1905–06, having regard to the months here compared (November to July, inclusive). The 100-bird pen of 1904–05 made a better record during the summer than the corresponding pens of 1905–06, but fall considerably below in winter production. In 1905–06 there was an environmental accident

TABLE I

MONTHLY DISTRIBUTION OF MEAN EGG PRODUCTION PER BIRD UNDER DIFFERENT BREEDING SYSTEMS

| Month | Weighted Mean Under Mass Selection | Best Comparable Year to 1913–14 of Similar-sized Flocks Under Mass Selection (1905–06 100-bird Pens) | Best Month in Any Year of Mass Selection, Any Size Flock | Year 1913–14 |
|---|---|---|---|---|
| November. | 4.63 | 5.38 | 6.45 (1904–05, 100-bird flock) | *10.76* |
| December. | 8.91 | 9.91 | 12.02 (1901–02, only 48 birds in small flocks) | *14.19* |
| January... | 11.71 | 13.27 | 15.21 (1901–02, only 48 birds in small flocks) | *13.88* |
| February.. | 10.87 | 13.39 | 14.46 (1905–06, 50-bird flocks) | *13.37* |
| March.... | 16.11 | 17.33 | 18.29 (1905–06, 50-bird flocks) | *19.22* |
| April..... | 15.85 | 16.48[7] | 18.50 (1901–02, only 48 birds in small flocks) | *18.44* |
| May..... | 13.92 | ——[8] | 17.02 (1902–03, 147 birds in small flocks) | *16.88* |
| June..... | 12.46 | 13.47[7] | 16.88 (1901–02, only 48 birds in small flocks) | *14.56* |
| July..... | 10.87 | 10.49[8] | 14.90 (1901–02, only 48 birds in small flocks) | *14.52* |

(overfeeding of green food) in the latter part of April. This adversely affected the May production. The 100-bird pens were more affected than the 50-bird pens. Consequently, in order to give every possible advantage to the earlier period of the work, I have taken the 50-bird pen averages for April, June and July and have graphically interpolated the figure for May in the diagram.

The data in Table I are set forth graphically in Fig. 1.

From the table and the diagram the following points are to be noted:

1. It is apparent that the laying in the part of the laying year covered by the statistics was distinctly better in 1913–14 than either the weighted mean of the whole period of mass selection, or than in the best comparable year of the earlier period.

2. The difference is somewhat more pronounced in respect of winter production (*i. e.*, the laying prior to March 1) than for any other cycle. Under the earlier plan of breeding the average winter production was 36.12 eggs. This production corresponds reasonably closely to the division point at 30 eggs between genetically high and

[7] Average from 50-bird pens of same year (1905–06). See text.

[8] Average omitted because of abnormal conditions. See text.

genetically mediocre winter producers which was used in the Mendelian analysis. In the year 1905–06 the mean winter production was 41.95 eggs. In 1913–14 the pro-

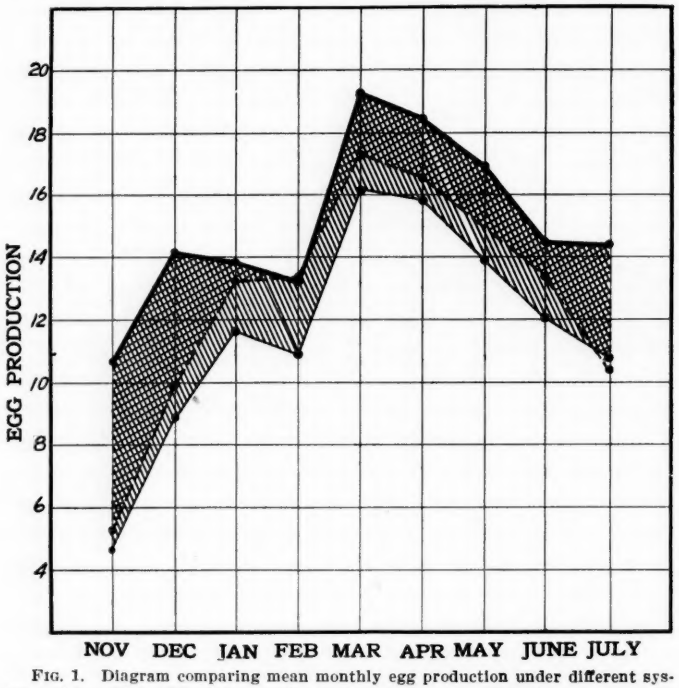


FIG. 1. Diagram comparing mean monthly egg production under different systems of breeding. The light continuous line gives the weighted means for the earlier years, the heavy continuous line the means for 1913–14, and the dotted line the means for 1905–06 100-bird pens. The cross-hatched area in comparison with the unruled area indicates in the increase of the 1913–14 averages over the earlier figures.

duction in the corresponding months was 51.20 eggs per bird.

3. It was shown by Pearl and Surface[9] that, on the average, a flock of hens produces 81.73 per cent. of their total annual yield between November 1 and August 1. Applying this figure to the 1913–14 nine-month total of 135.82 eggs, we get for the probable production of this

[9] "Biometrical Study of Egg Production in the Domestic Fowl. II. Seasonal Distribution of Egg Production," U. S. Dept. of Agr., B. A. I. Bull. 110, Pt. II, p. 89, 1911.

flock of 192 birds from November 1 to November 1 a total of 166.18 eggs. This value, as a matter of fact, is very close to the average production per bird of those (53) out of the 192 which were kept over for experimental purposes a second year. The corresponding total for the weighted mean annual production over the whole period is 128.86.

4. Taking the artificial year given in next to the last column of the table it is seen that in 1913–14, with 125-bird flocks, the November, December and March averages were higher than the highest made in the corresponding months during the mass-selection period, regardless of size of flock or other conditions. The April, May and July averages in 1913–14 were substantially equal to the highest made in the corresponding months under mass-selection. The highest January, February and June averages in the mass-selection period were from 1 to 2 eggs higher than the corresponding months in 1913–14. Taking the totals of the whole 9-month period compared, we have for the artificial year, made up of the highest mean monthly production under mass selection for each month regardless of the year or the flock size, *a total of 133.73 eggs per bird, while that for the single year 1913–14 is 135.82.*

Another comparison, which brings out some additional facts, is set forth in Table II. Any bird laying 18 or more eggs per month in the months November, December, January and February may certainly be regarded as a high winter producer. The proportion of such high producers in the whole flock gives valuable additional information to that furnished by the means, since the monthly egg production variation curves are distinctly skew. The

TABLE II

SHOWING PROPORTION OF FLOCK LAYING 18 OR MORE EGGS IN THE SPECIFIED MONTHS

| Month | Total Flocks 1899–1907, Per Cent. | 100-bird Flocks 1905–1906, Per Cent. | Flock of 1913–1914, Per Cent. |
|---|---|---|---|
| November | 7.0 | 5.5 | 26.0 |
| December | 19.0 | 30.2 | 47.4 |
| January | 24.2 | 36.3 | 42.2 |
| February | 22.6 | 36.3 | 31.8 |

mean and median do not coincide. In Table II is shown the percentage of the whole flock laying 18 or more eggs in the months specified.

This table shows in an even more striking way than the means in Table I the marked difference between the flocks of the present time and those of the earlier years. In 1913–14 nearly half the flock laid 18 or more eggs each during December and January.

The data presented in this paper establish, I think, the following facts:

1. There is a marked difference in the average production per bird of Barred Plymouth Rock pullets of the Maine Station strain at the present time, as compared with what obtained in the earlier trap-nesting work of the Station described by Pearl and Surface (*loc. cit.*).

2. This difference is in the direction of *a substantially higher mean flock production at the present time.*

3. The increase in flock production is most pronounced in respect to winter production.

The most probable explanation of the above results appears to the writer to be that the plan of breeding now followed is more nearly in accord with the biological facts regarding the inheritance of fecundity than was the plan followed in the earlier years.

The reasons for this opinion, while not constituting complete proof of the suggested explanation, certainly make a strong body of evidence in its favor. They are, summarily stated:

(*a*) That the increases in flock productivity have been synchronous with changes in breeding practise.

(*b*) That the increases give every indication of being permanent, there having been no tendency towards a decline in flock productivity since 1908, when the simple mass selection was stopped and breeding begun on a progeny-test basis.

(*c*) That there have been no changes in management or environmental circumstances synchronous with the increases in flock production and capable of accounting for them. The hens are housed to-day in the same houses

that they were in 1904; are fed substantially the same feed, the only modification of the ration having been in the direction of one *less* stimulating to production than the one formerly used; are hatched in the same sort of incubators; reared in the same yards, etc.

(*d*) That the most marked gains have been in that cycle of production (winter laying) to which especial attention was paid in the breeding.

(*e*) That when analyzed in terms of individual matings the results obtained in egg production have been the results to be expected on the Mendelian hypothesis of the inheritance of this character earlier set forth, with only minor exceptions for which the explanation is in nearly all cases apparent.

## II. An Independent Confirmation of the Sex-linkage of the Factor for High Fecundity

Besides the results with large flocks which have followed the practical application of the Mendelian hypothesis of fecundity inheritance at this Station, numerous poultrymen in various parts of the world have obtained similar results. Several instances of this sort might be cited from private correspondence. The writer has felt, however, that such cases really contributed nothing new in principle, and that therefore there was no special need of calling attention to them.

There lately appeared, however, in an English poultry paper, a note which seemed to me to be of interest on several grounds. In the first place, it is evident that the writer, Mr. E. N. Steane, is a careful observer, and an experienced poultryman. In the second place, his observations on inheritance of egg producing ability appear to be, from his point of view, entirely original and uninfluenced by any earlier work.

The parts of Mr. Steane's note[10] which are pertinent in the present connection are these:

[10] Steane, E. N., "The Production of 'Best Layers,'" *The Feathered World* (London), Vol. 52, p. 285, 1915.

My own experience, and that of many other breeders, tends to show that the birds hatched from high pedigree hens are not such prolific layers as those hatched from healthy hens of an indifferent laying strain mated to high pedigree cockerels.

For three or four seasons I bred from two-year-old white Leghorn hens of a gold-medal laying strain mated to a cockerel of equally-good descent, and the results, to my mind, were disappointing, and did not yield an adequate profit on the money spent. The pullets were less prolific than their parents, and inclined to be delicate and more or less undersized, while the percentage of fertile eggs was lessened.

Then by a lucky chance one season I had not enough eggs from a pen of Rhode Island Reds to fill up an incubator, and I made up the deficiency from a pen of good-sized healthy Leghorn hens of no particular laying strain mated to a pedigree cockerel. Practically every egg from this pen was fertile, the chickens proved strong, and the results seemed in every way satisfactory.

This, of course, led to my systematic mating of healthy, well-grown birds of indifferent laying strain to high pedigree cockerels, with very successful results. The fertility of the eggs was extremely satisfactory, the chickens turned out strong and healthy, and the pullets on arriving at maturity were highly prolific layers, each pullet averaging 200 eggs and over during the first twelve months, as against about 130 from the pullets of the high pedigree hens, many of whom also died off. In the second year the birds did equally well, the number of eggs being maintained and all being of a good size.

Later, I tried the result of mating high pedigree hens to a healthy cockerel of no special laying strain, but without success, the chickens being healthy, but the laying results much below the average, so that nothing was to be gained by further trials in that direction.

While being quite aware that many breeders do not agree with my conclusions, and that a great deal also depends on the condition and environment of the birds—prolificacy being always greatly improved by the birds having a free range, I am myself firmly convinced that such mating makes for the production of best layers. All my experiments were, of course, carried out under the same conditions in each case, the birds being kept in runs of 20 yards by 10, on well-drained, sandy soil, with a house and scratching shed attached, and fed on the same diet as that adopted in the recent laying competitions.

It is evident that Mr. Steane's experience was exactly parallel to the results of the present writer's investigations reported in earlier papers. High producing females did not transmit that quality directly to their daughters. The character is sex-linked.

The only point of difference is that noted in the second

paragraph of the quotation, and I think that the explanation of the discrepancy there is contained in the closing words of the paragraph where Mr. Steane says:

> The pullets were . . . inclined to be delicate and more or less undersized, while the percentage of fertile eggs was lessened.

This would indicate that other causes besides the breeding operations were working to bring about a poor physiological condition of the progeny, which is of course inconsistent with high productivity. Lowered fertility of eggs is one of the best indicators of reduced vitality which can be found.

We appear to have, in this case, a rather complete independent confirmation by a practical poultryman of one of the present writer's chief results in regard to the inheritance of fecundity.

## III. Summary

In this paper it has been shown that:

1. There is a marked difference in average egg production per bird of Barred Plymouth Rock pullets of the Maine Station strain at the present time as compared with what obtained during the period of simple mass-selection for this character.

2. This difference is in the direction of a substantially higher mean production at the present time, when tested on flocks of large size.

3. The increase in flock average productivity is most pronounced in respect to winter production, which is the laying cycle to which especial attention has been given in the breeding.

4. The cause of this increase in flock productivity appears, with a degree of probability which is very high and amounts nearly to certainty, to be that the method of breeding the stock now followed is more closely in accord with the mode of inheritance of fecundity than was the simple mass-selection practised in the earlier period.

5. The result announced in earlier papers that high fecundity is a sex-linked character, for which the female is heterozygous, has been confirmed by practical poultrymen in their breeding operations.

## SHORTER ARTICLES AND DISCUSSION

### THE APPEARANCE OF KNOWN MUTATIONS IN OTHER MUTANT STOCKS

In *Drosophila ampelophila* the reappearance of known mutations in stocks that appear to be uncontaminated is a not unfamiliar occurrence, but we discount all such cases unless in some way the occurrence can be controlled, because the chance of contamination even with extreme care might be claimed to be greater than the chance of mutating.

In the stock of sepia-eyed flies a few individuals with very pale (yellowish red) eyes appeared. Sepia eyes are very dark or black brown in color. So that the flies with the new eye color stood out conspicuously amongst the dark-eyed sepias.

From the color of the eye it was suggested that it might be vermilion-sepia. If this were the case it should give, when bred to vermilion flies, vermilion-eyed offspring, because the factor for vermilion would be common to both stocks and the stock vermilion would carry the normal (dominant) allelomorph of sepia. When the test was made the offspring were vermilion. These $F_1$'s inbred gave in $F_2$ 122 vermilion to 39 vermilion sepia, approximately 3:1, which is the expectation for one factor difference. The result shows that a mutation to vermilion eyes had taken place in stock that had already sepia eyes. The resulting flies were the double recessive vermilion sepia.

That the result is not due to contamination is evident, for had a vermilion-eyed fly got into the sepia stock it would have produced red-eyed (wild type) females or vermilion males. As no red- or vermilion-eyed flies were present this explanation is excluded.

A similar mutation took place in stock having purple eyes. Like sepia the eye color of these flies is dark but in this case has a distinct purplish-red tinge. Among the offspring from a cross of a female heterozygous for purple with a pure purple male a fly with very pale orange-colored eyes appeared. This fly, which was a male, was also conspicuously unlike the remainder of the red or purple offspring of this pair.

It was at first thought possible that this was the appearance of a mutation to cherry in a mutant stock. If this had been the case we should expect that in a cross to a cherry female all of $F_1$ offspring would be cherry. The test showed instead all cherry

males and all red females—the normal dominant color. This proved then that the new fly did not contain the factor for cherry.

It was then suggested that this was a second case of the appearance of vermilion in mutant stock. Since the fly was a male several matings were possible, and it was therefore crossed to a vermilion female. As in the previous case if vermilion were common to both stocks, the offspring should be all vermilion. This condition was actually found in all the $F_1$ offspring of this second cross, and the $F_2$'s gave vermilion and the orange-eyed fly—now shown to be vermilion purple—in approximately 3:1 classes. This demonstrated that a mutation to vermilion had taken place in a fly already having purple eyes, for as the cherry cross indicated, the pure mutant stock contains the normal dominant allelomorph to every factor except the one it shows.

In order to demonstrate that the purple factor was still present unchanged in the germ cells of the double recessive (vermilion purple) fly, the original male was also mated to pure purple-eyed stock. As in the other cases if both parents contain the factor for purple all the offspring should be purple. This was the actual result obtained, and it proves the original mutant to have been the double recessive vermilion purple.

In this case also it is not possible that the result could have been due to contamination. This would indeed be highly improbable when the original parents were a single isolated pair, the female of which was a virgin when first mated. But even had a vermilion male been able to mate with the heterozygous female only red-eyed flies could have been produced. The vermilion purple combination could not occur because the germ cells of each animal carry the normal dominant allelomorph of the mutation in the other.

Our results in these two cases show that mutations within other mutant stocks occur, and they also indicate that in the case of vermilion we have a mutation which has recently reappeared twice.

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## THE EVENING PRIMROSE VARIETIES OF DE VRIES

No explanation of the "variation" of heterozygous plants had presented itself until Mendel went back to the haploid generation, and referred the differences in the progeny of heterozygotes to the segregation of differences in the pollen-grains and embryo-sacs from which the plants had arisen. He dealt, however, with plants

in which all the young pollen-grains and embryo-sacs had presumably an equal chance to mature.

Perhaps a parallel state of affairs exists to-day. De Vries and others have brought to light, in the progeny of Œnotheras, a certain amount of "variation," by no means so striking as can be seen in the progeny of many variety or species crosses, but remarkable chiefly because it could not be explained. In my opinion, this "variation" can perhaps be explained by going back, as Mendel did, to the haploid generation. We may, I think, presume that there are certain genetic factors concerned with the development of the young microspore into a complete pollen-grain, and the young megaspore into a normal embryo-sac. If the plants are heterozygous for one or more of these factors, we get definite ratios of normal and aborted pollen-grains, or normal and aborted embryo-sacs. We thus have a population of haploid individuals (microspores or megaspores) which show segregation into viable and non-viable. We may expect ratios of normal and aborted haploid individuals of 1:1; 1:3; 1:7; 1:15, etc., applying to either pollen-grains or to embryo-sacs, or to both. When we thus have heterozygosity of one or more factors essential for the development of the individuals of the haploid generation, the laws and ratios for certain characters of the diploid generation may become very different.

According to Geerts, who seems to have made the only accurate study of the point, the typical *Œnothera lamarckiana* aborts one half its microspores (two from each tetrad) and one half its embryo-sacs. The simplest hypothesis demands two factors, one essential for the development of pollen-grains, and one for the development of embryo-sacs, which factors show complete (or nearly complete) repulsion. Then, of course, the offspring will be permanently heterozygous for these two factors, and also more or less for any other factors which may be linked with them. (Linkage has been shown to exist, I think, in all plants where it has been looked for.) With more than two such factors heterozygous, the aborted pollen-grains or embryo-sacs may increase, and the ratios in the progenies of crosses become altered. Hence, in my opinion, a promising step towards the investigation of inheritance in Œnotheras is the correct determination of the ratios of aborted and normal pollen-grains and embryo-sacs in the different "varieties" of *Œnothera lamarckiana*, and related species.

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